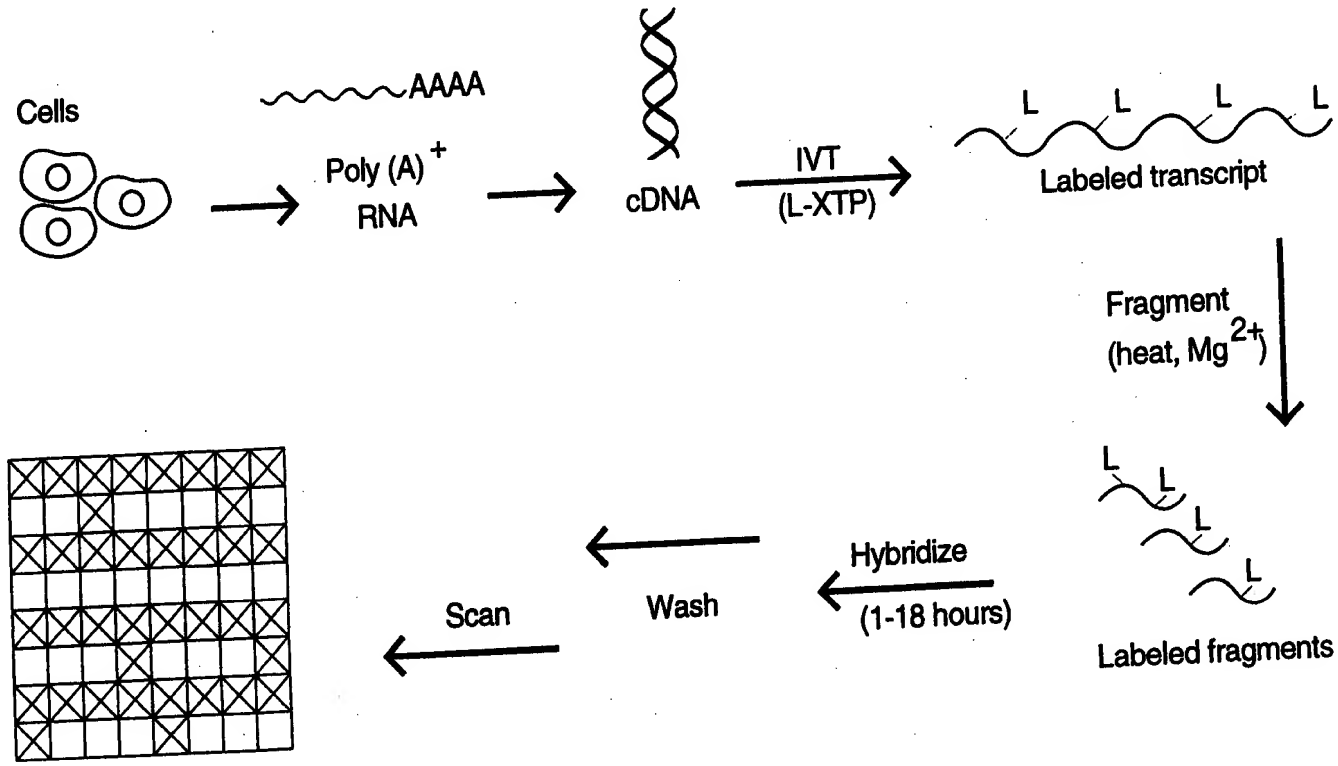

Figure 1

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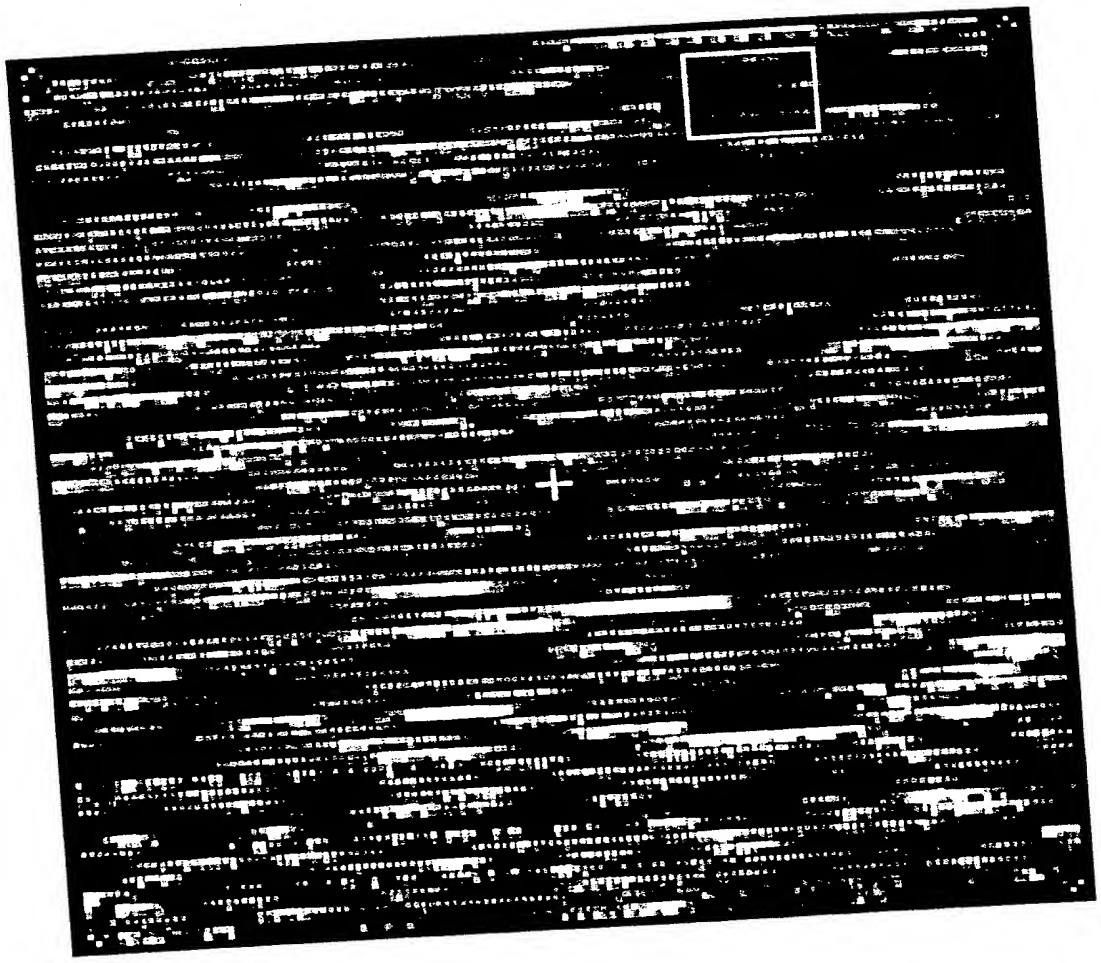


Figure 2a

Figure 2b

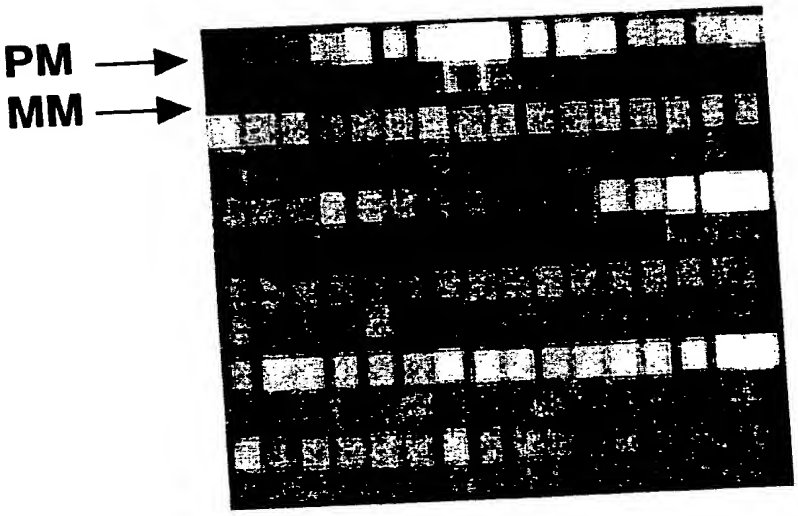
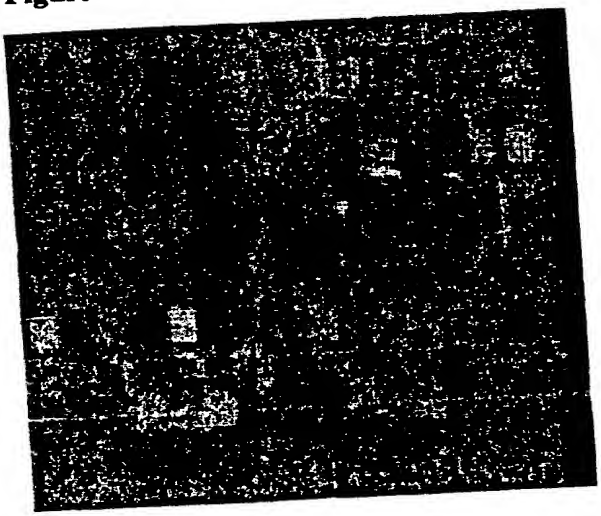


Figure 2c



SUBSTITUTE SHEET (RULE 26)

Hybridization Signal vs Target Concentration

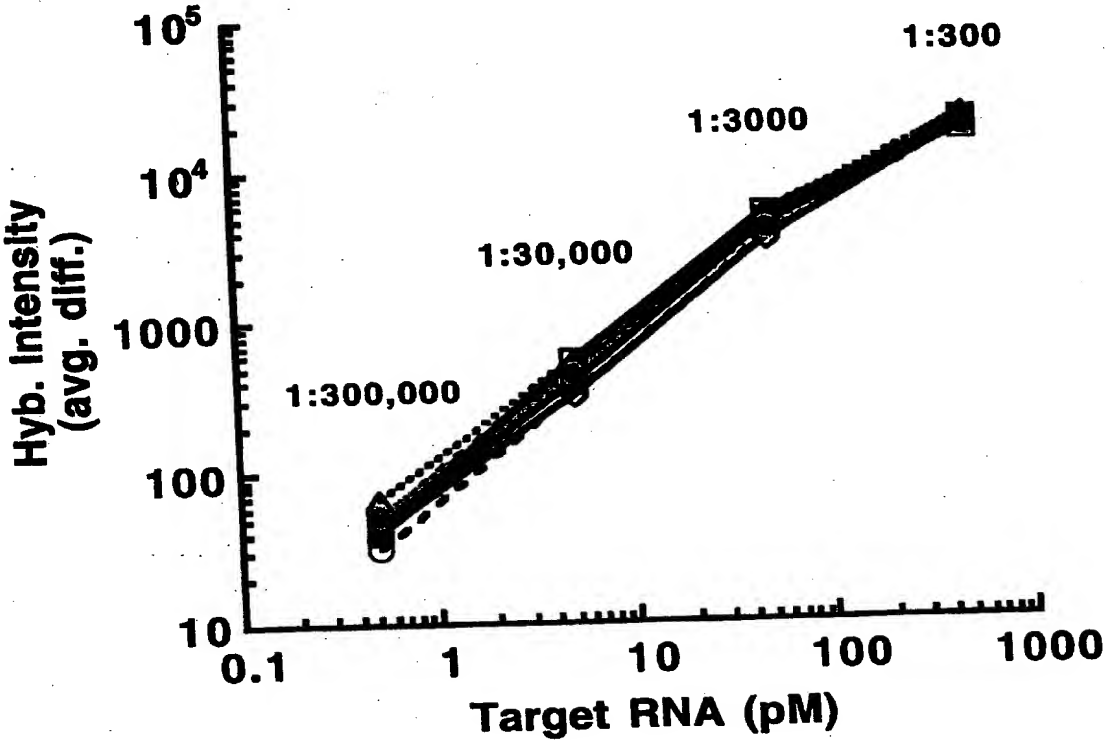


Figure 3

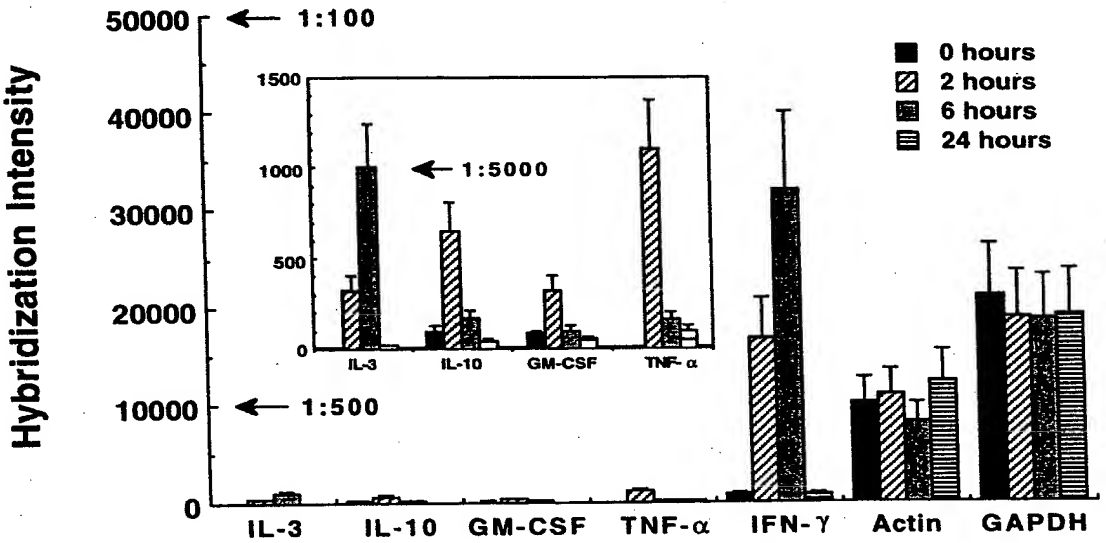

Hybridization Intensity
50000
40000
30000
20000
10000
0
1:100
1:500
1:5000
1500
1000
500
0
IL-3
IL-10
GM-CSF
TNF-α
0 hours
2 hours
6 hours
24 hours
IL-3
IL-10
GM-CSF
TNF-α
IFN-γ
Actin
GAPDH

Figure 4

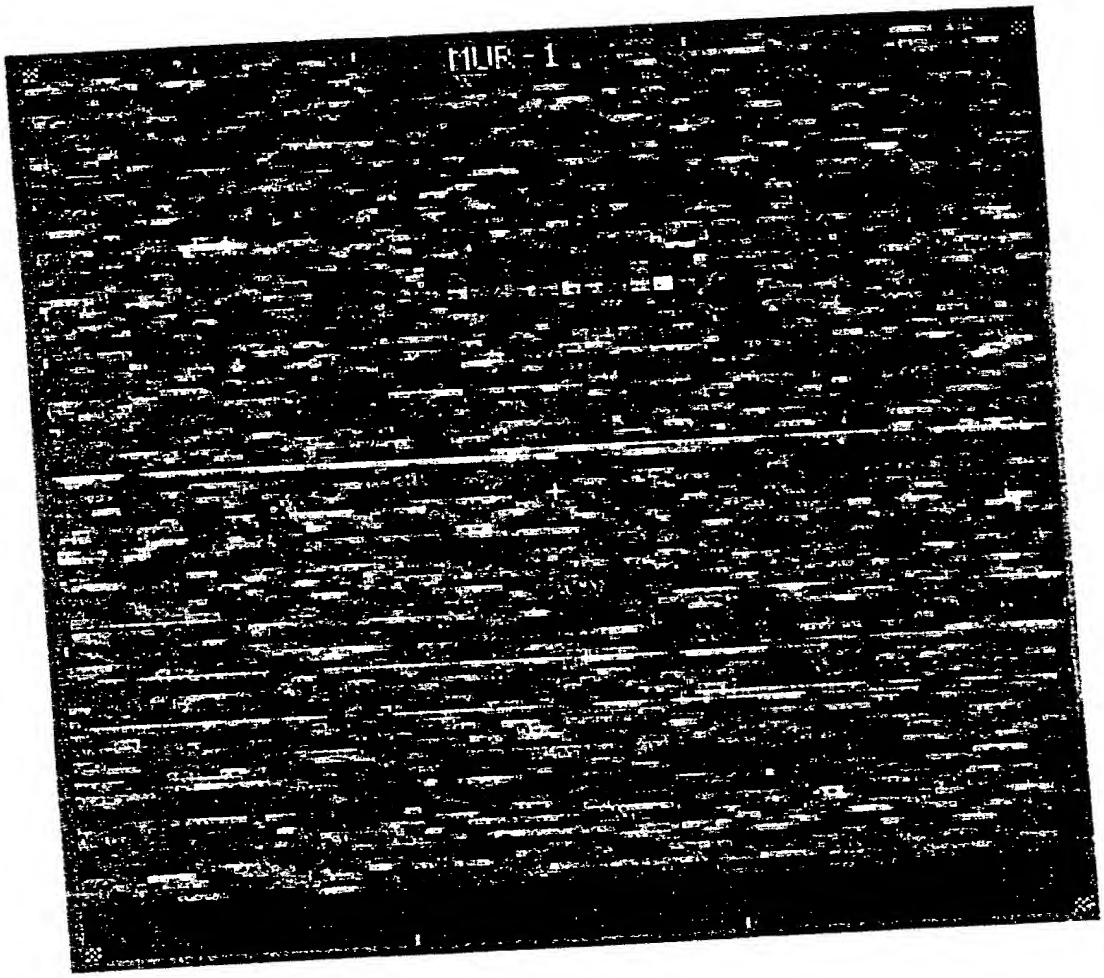


Figure 5

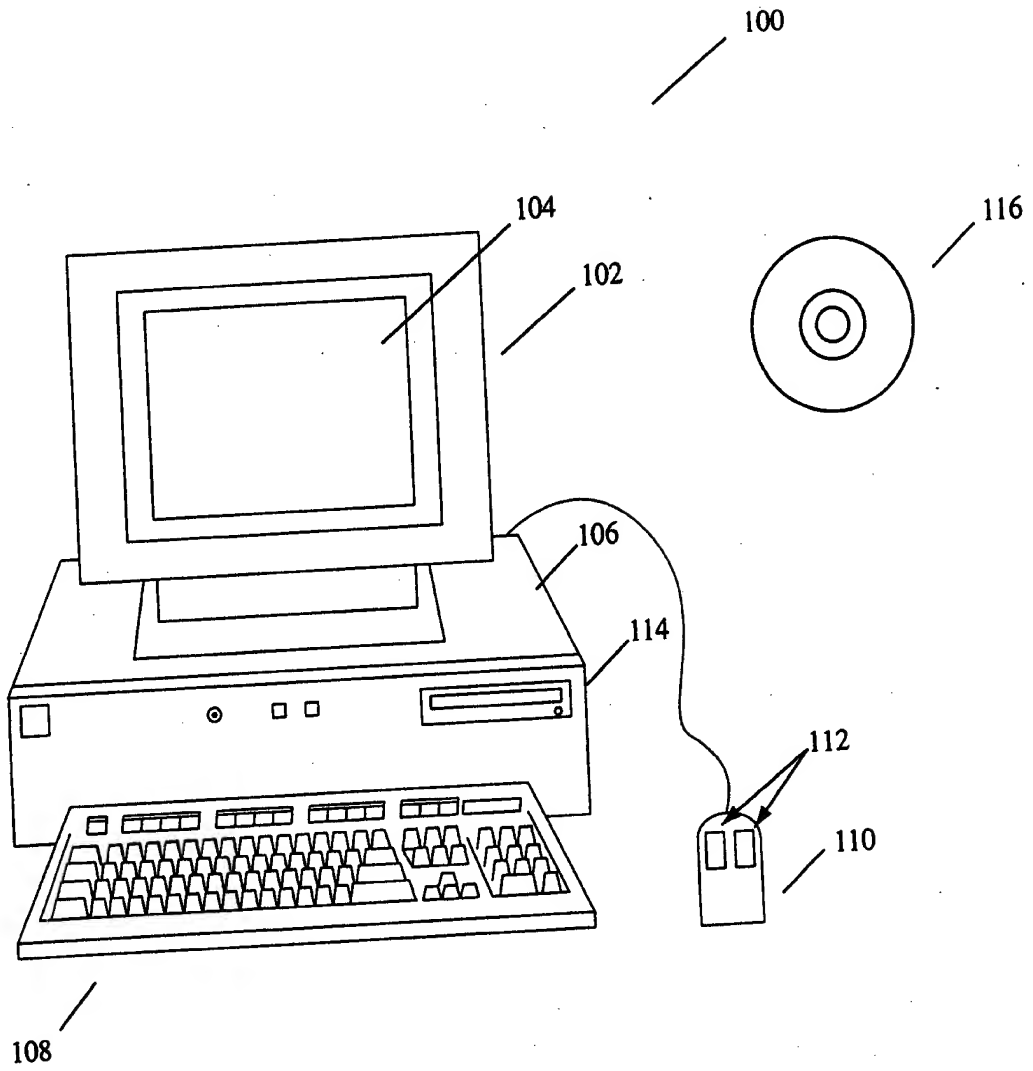


Figure 6

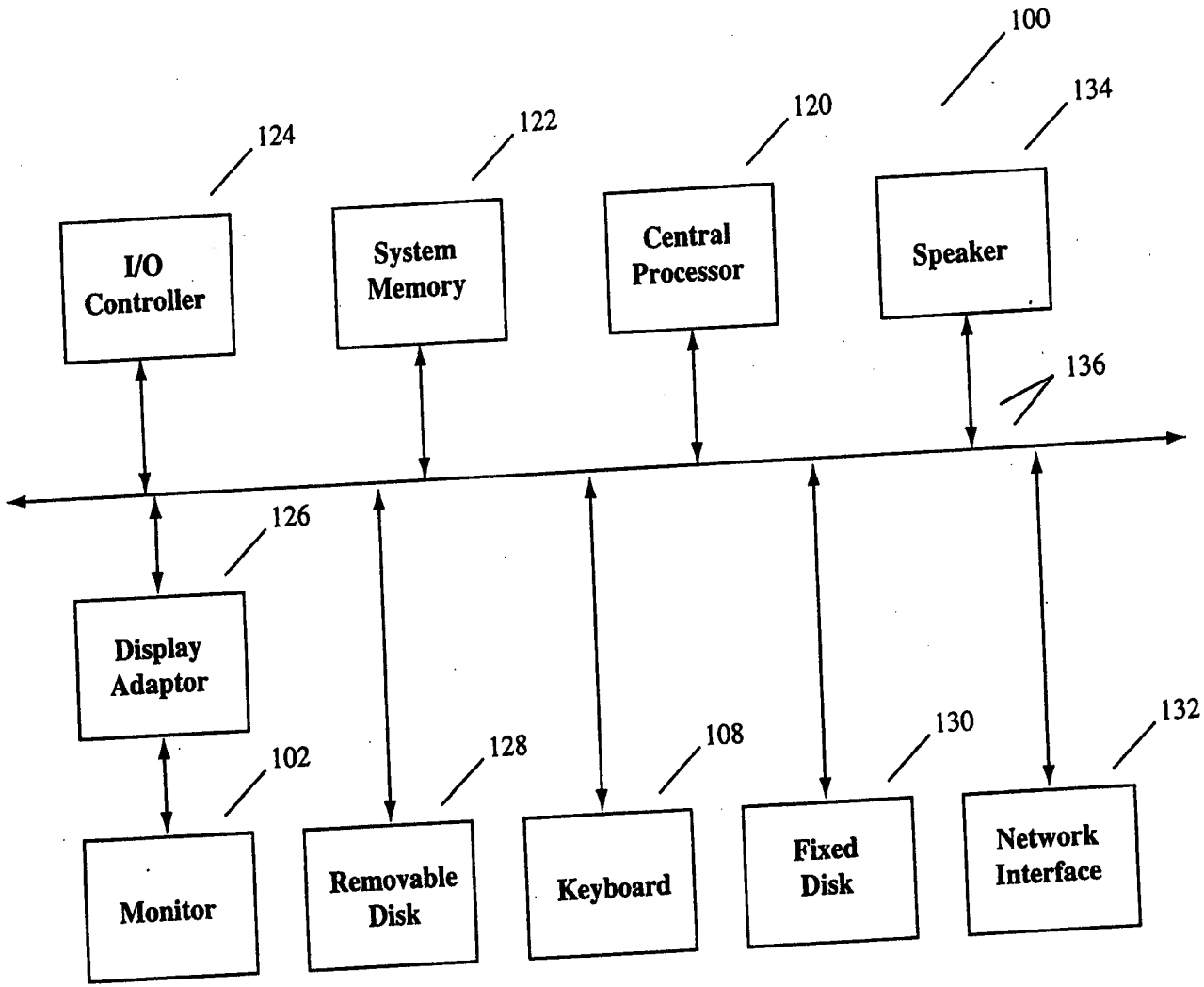

100
124
122
120
134
I/O Controller
System Memory
Central Processor
Speaker
136
126
Display Adaptor
102
128
108
130
132
Monitor
Removable Disk
Keyboard
Fixed Disk
Network Interface

Figure 7

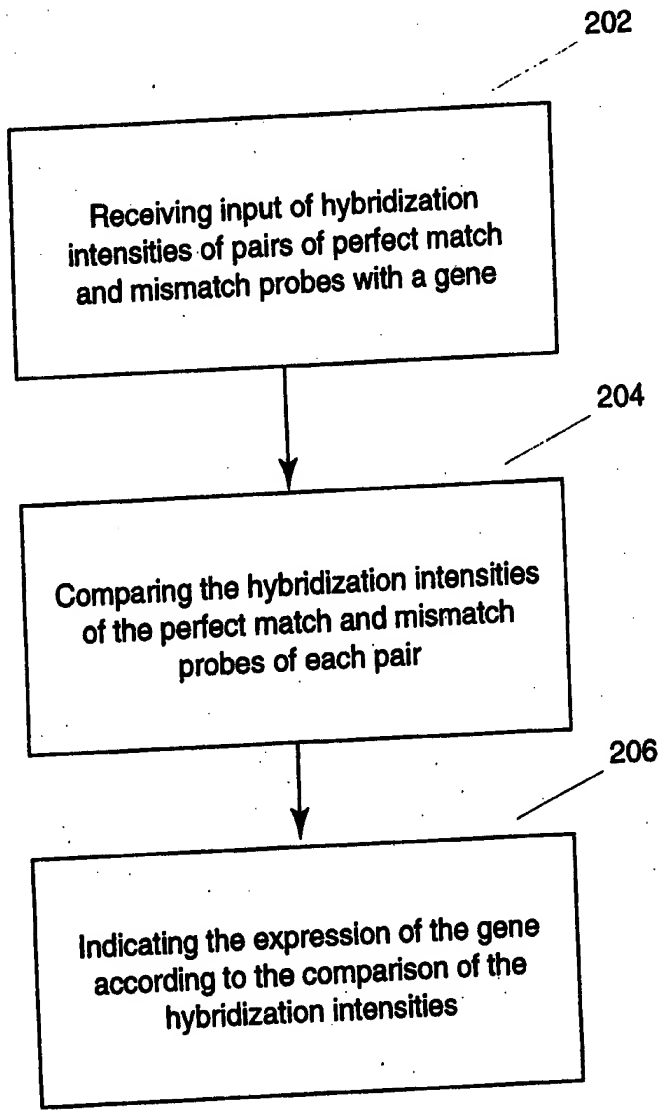

202
Receiving input of hybridization intensities of pairs of perfect match and mismatch probes with a gene
204
Comparing the hybridization intensities of the perfect match and mismatch probes of each pair
206
Indicating the expression of the gene according to the comparison of the hybridization intensities

Figure 8

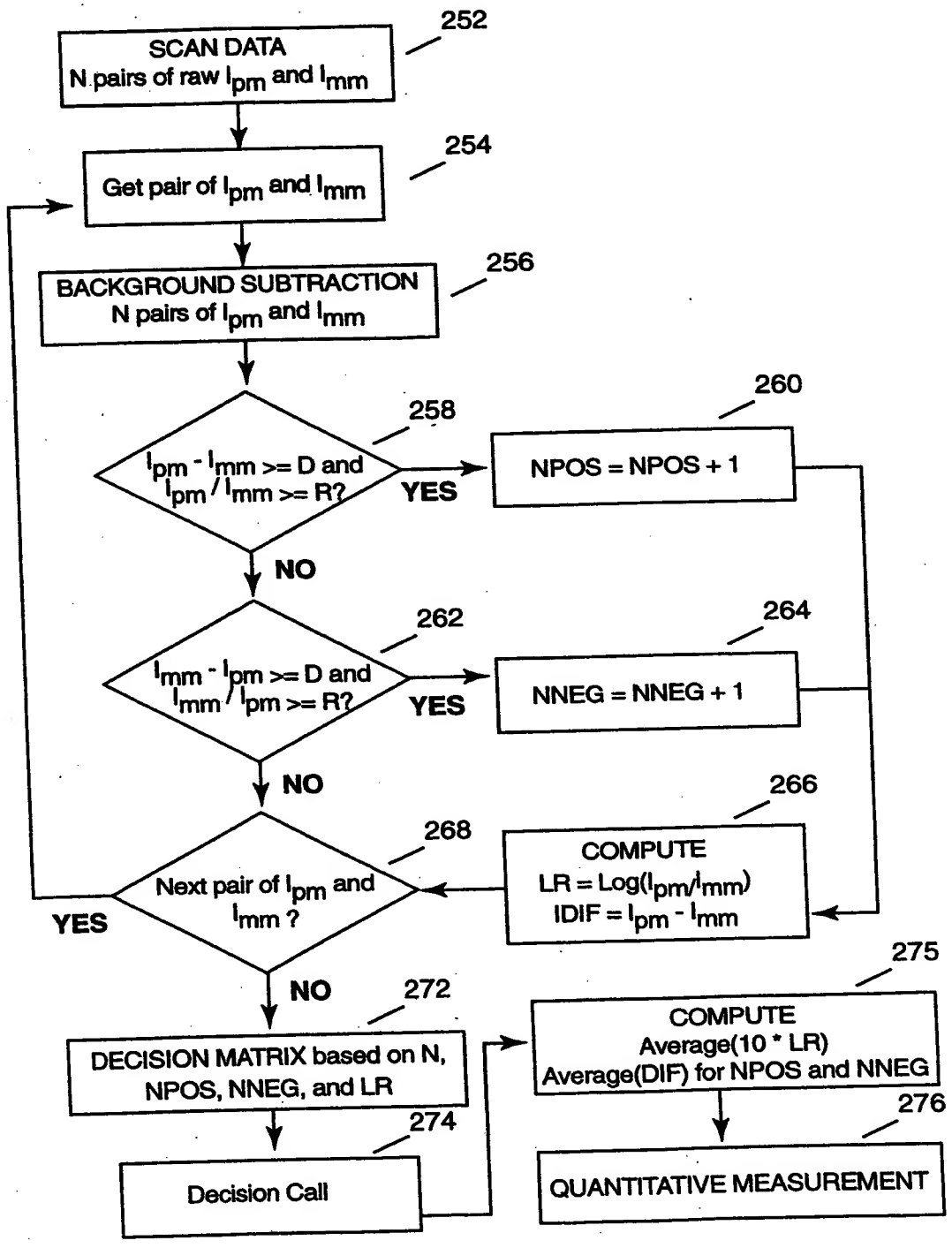

SCAN DATA
N pairs of raw I_{pm} and I_{mm}
252
Get pair of I_{pm} and I_{mm}
254
BACKGROUND SUBTRACTION
N pairs of I_{pm} and I_{mm}
256
$I_{pm} - I_{mm} >= D$ and $I_{pm} / I_{mm} >= R$?
258
YES
NPOS = NPOS + 1
260
NO
$I_{mm} - I_{pm} >= D$ and $I_{mm} / I_{pm} >= R$?
262
YES
NNEG = NNEG + 1
264
NO
COMPUTE
$LR = Log(I_{pm}/I_{mm})$
$IDIF = I_{pm} - I_{mm}$
266
Next pair of I_{pm} and I_{mm} ?
268
YES
NO
DECISION MATRIX based on N, NPOS, NNEG, and LR
272
Decision Call
274
COMPUTE
Average(10 * LR)
Average(DIF) for NPOS and NNEG
275
QUANTITATIVE MEASUREMENT
276

Figure 9

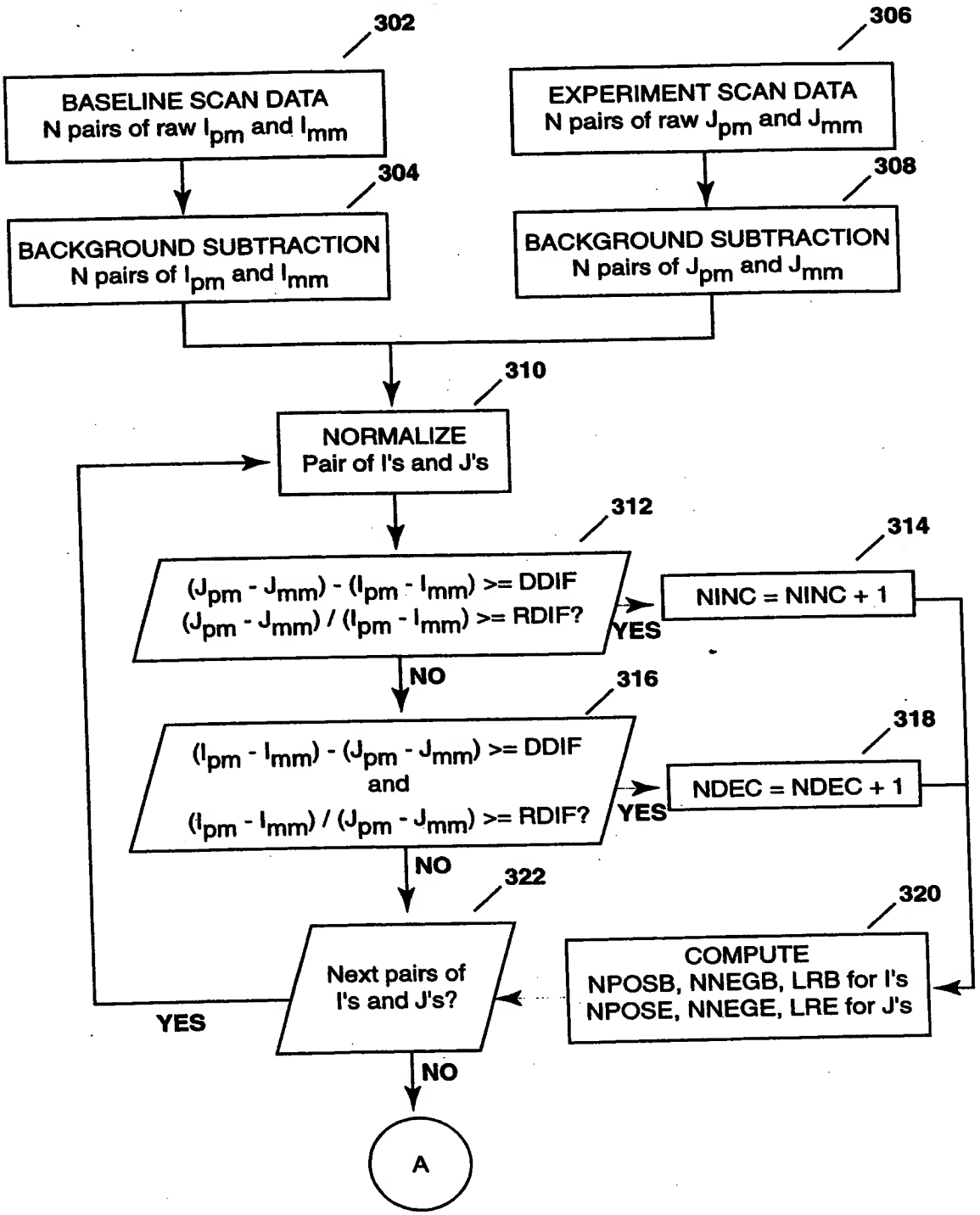

302
BASELINE SCAN DATA
N pairs of raw I_{pm} and I_{mm}
304
BACKGROUND SUBTRACTION
N pairs of I_{pm} and I_{mm}
306
EXPERIMENT SCAN DATA
N pairs of raw J_{pm} and J_{mm}
308
BACKGROUND SUBTRACTION
N pairs of J_{pm} and J_{mm}
310
NORMALIZE
Pair of I's and J's
312
$(J_{pm} - J_{mm}) - (I_{pm} - I_{mm}) >= DDIF$
$(J_{pm} - J_{mm}) / (I_{pm} - I_{mm}) >= RDIF?$
YES
314
NINC = NINC + 1
NO
316
$(I_{pm} - I_{mm}) - (J_{pm} - J_{mm}) >= DDIF$
and
$(I_{pm} - I_{mm}) / (J_{pm} - J_{mm}) >= RDIF?$
YES
318
NDEC = NDEC + 1
NO
322
Next pairs of
I's and J's?
YES
320
COMPUTE
NPOSB, NNEGB, LRB for I's
NPOSE, NNEGE, LRE for J's
NO
A

Figure 10a

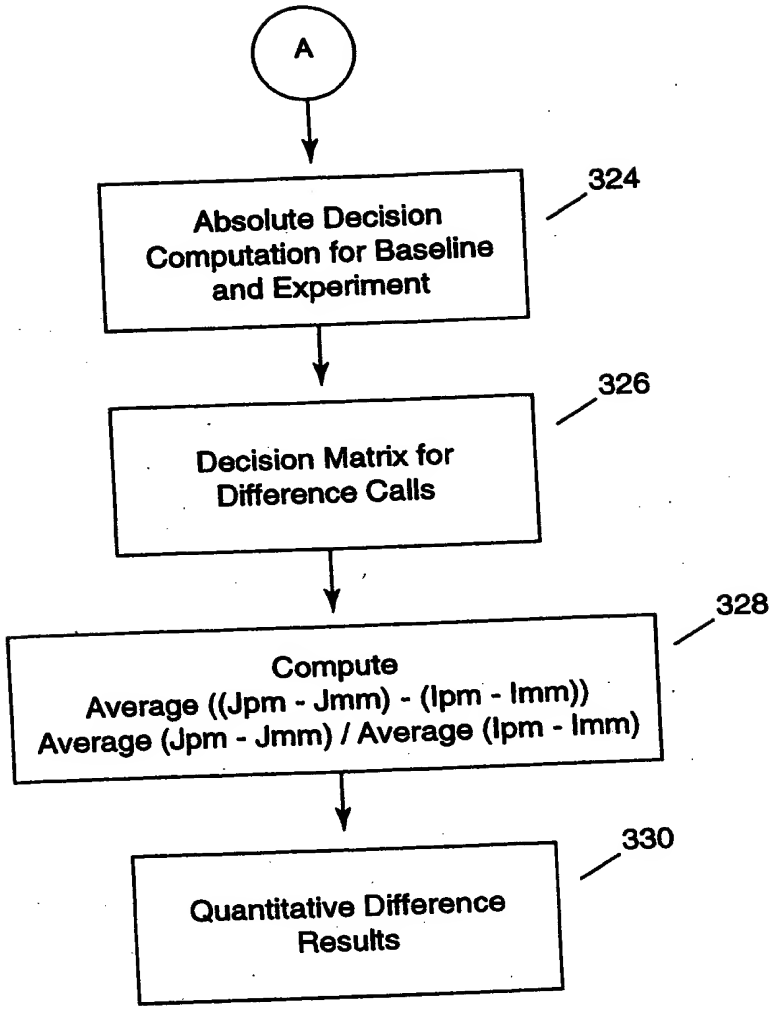

A
Absolute Decision Computation for Baseline and Experiment
324
Decision Matrix for Difference Calls
326
Compute
Average ((Jpm - Jmm) - (Ipm - Imm))
Average (Jpm - Jmm) / Average (Ipm - Imm)
328
Quantitative Difference Results
330

Figure 10b

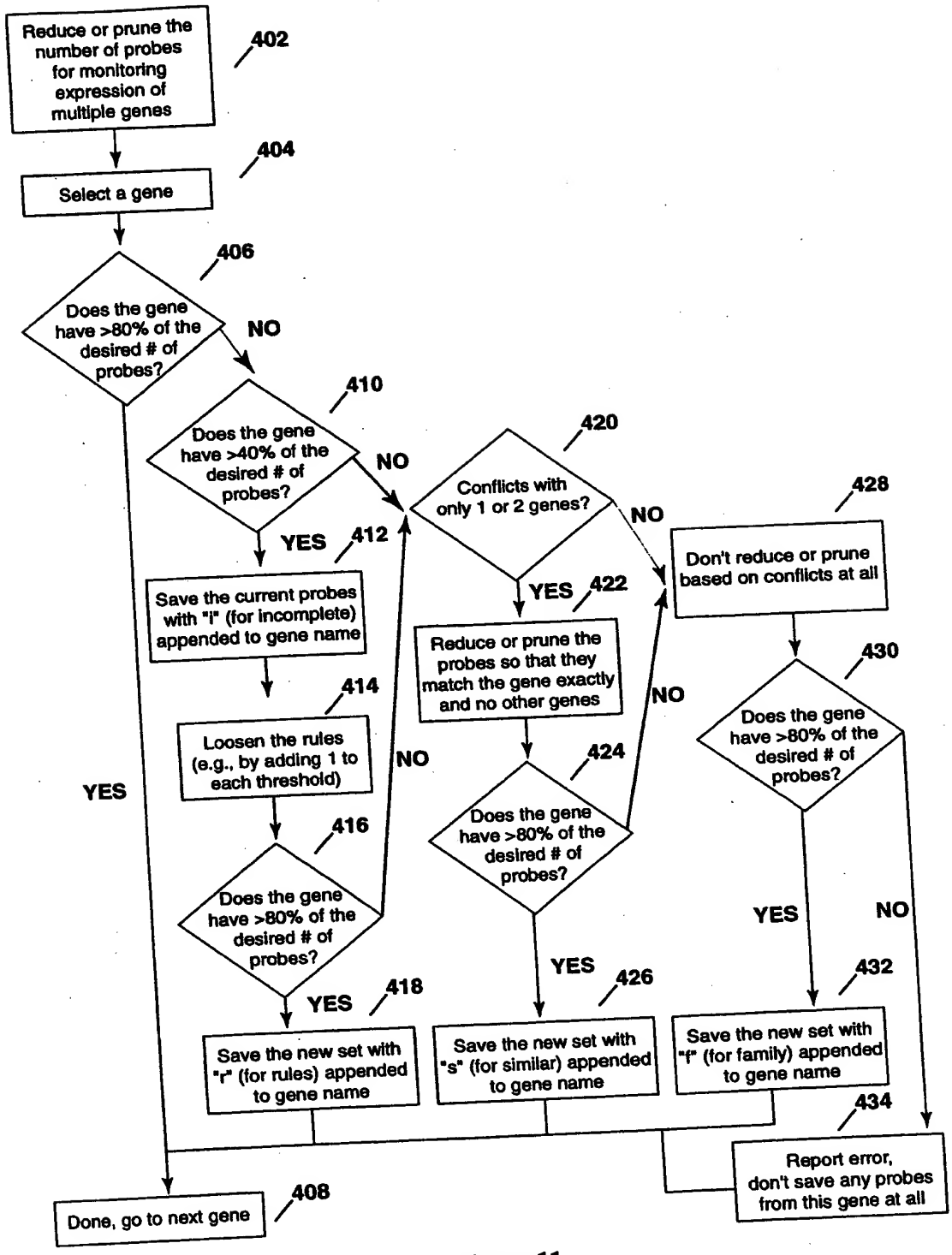

Reduce or prune the number of probes for monitoring expression of multiple genes
402
Select a gene
404
Does the gene have >80% of the desired # of probes?
406
NO
YES
Does the gene have >40% of the desired # of probes?
410
NO
YES
412
Save the current probes with "i" (for incomplete) appended to gene name
Loosen the rules (e.g., by adding 1 to each threshold)
414
Does the gene have >80% of the desired # of probes?
416
NO
YES
Save the new set with "r" (for rules) appended to gene name
418
Conflicts with only 1 or 2 genes?
420
NO
YES
422
Reduce or prune the probes so that they match the gene exactly and no other genes
Does the gene have >80% of the desired # of probes?
424
NO
YES
Save the new set with "s" (for similar) appended to gene name
426
Don't reduce or prune based on conflicts at all
428
Does the gene have >80% of the desired # of probes?
430
YES
NO
Save the new set with "f" (for family) appended to gene name
432
Report error, don't save any probes from this gene at all
434
Done, go to next gene
408

Figure 11

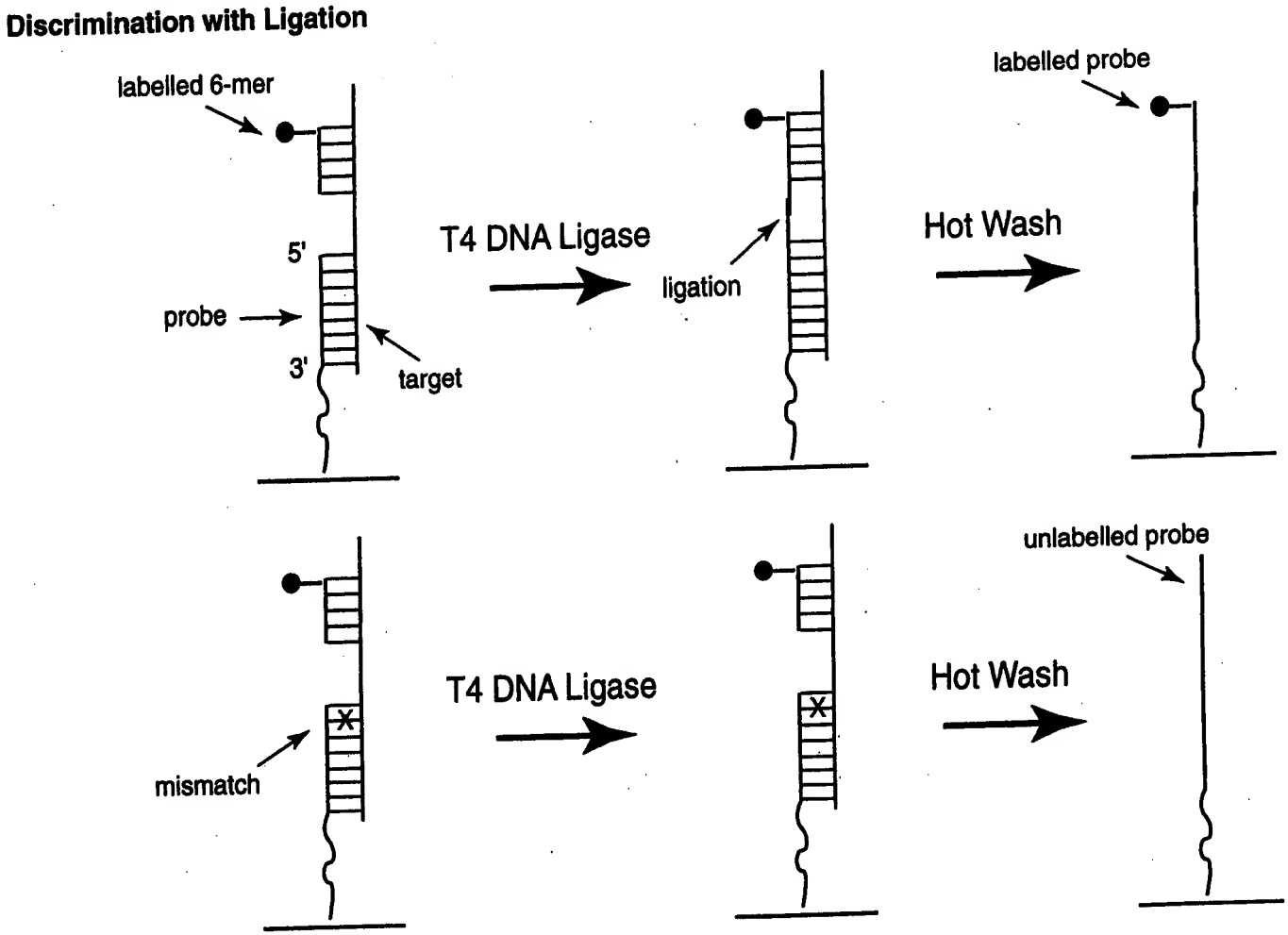

Discrimination with Ligation
labelled 6-mer
5'
probe
3'
target
T4 DNA Ligase
ligation
Hot Wash
labelled probe
mismatch
T4 DNA Ligase
Hot Wash
unlabelled probe

Figure 12

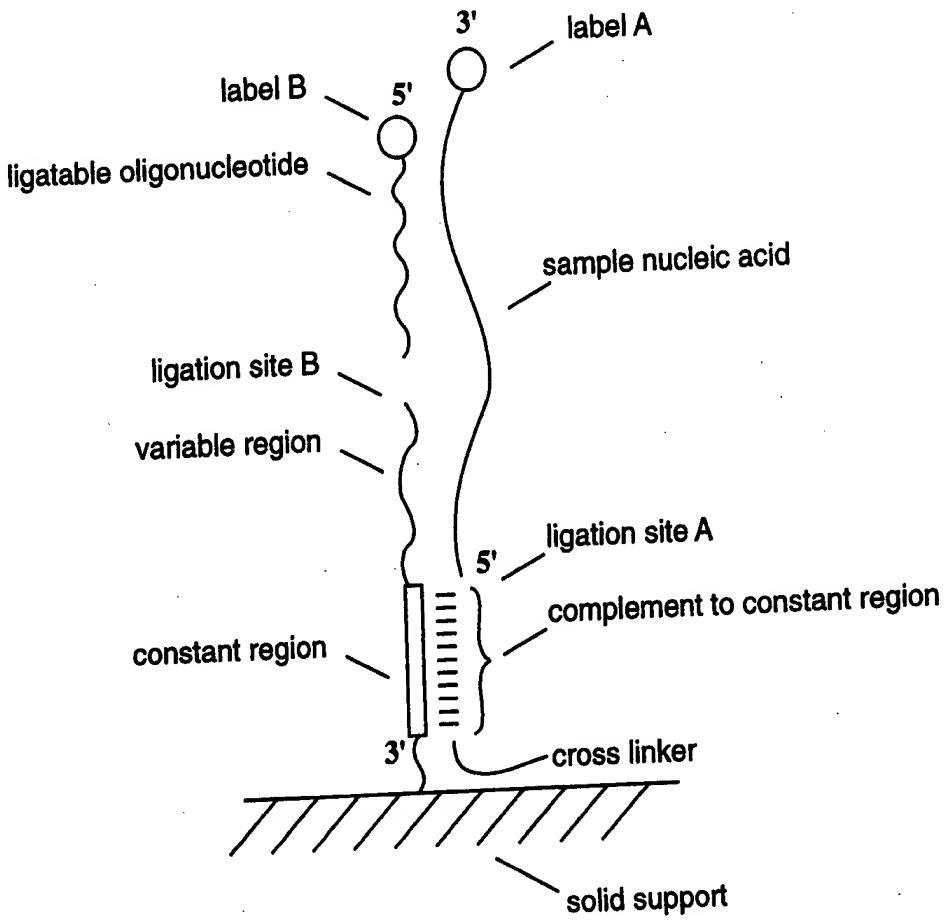

3'
label A
label B
5'
ligatable oligonucleotide
sample nucleic acid
ligation site B
variable region
ligation site A
5'
complement to constant region
constant region
3'
cross linker
solid support

Figure 13a

T0E790 2240860

Figure 13b

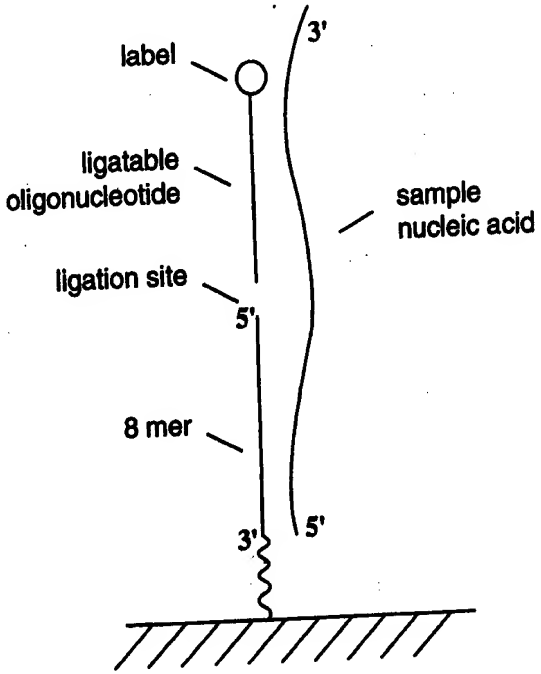
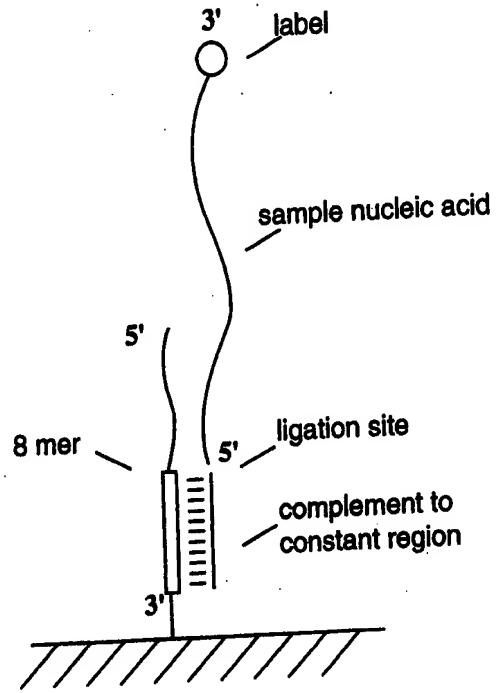


Figure 13c



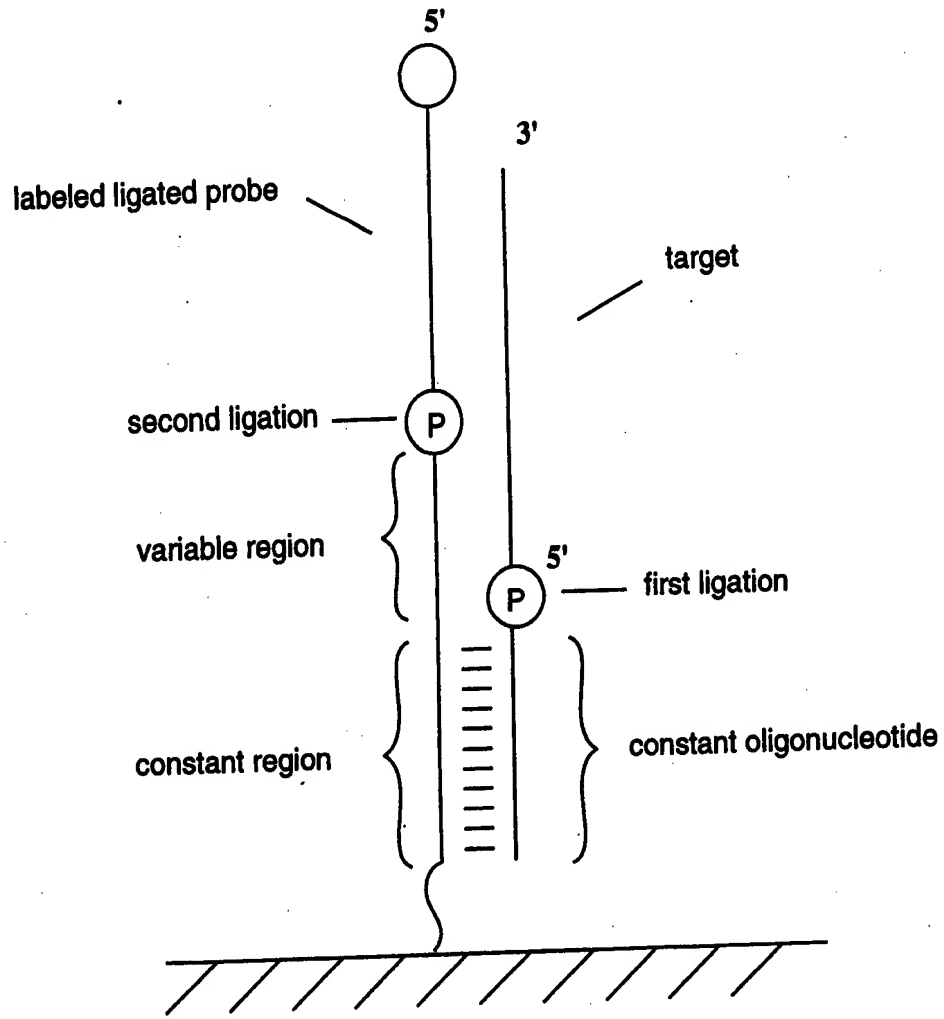

5'
3'
labeled ligated probe
target
second ligation
P
variable region
5'
P
first ligation
constant region
constant oligonucleotide

Figure 13d

Figure 14a

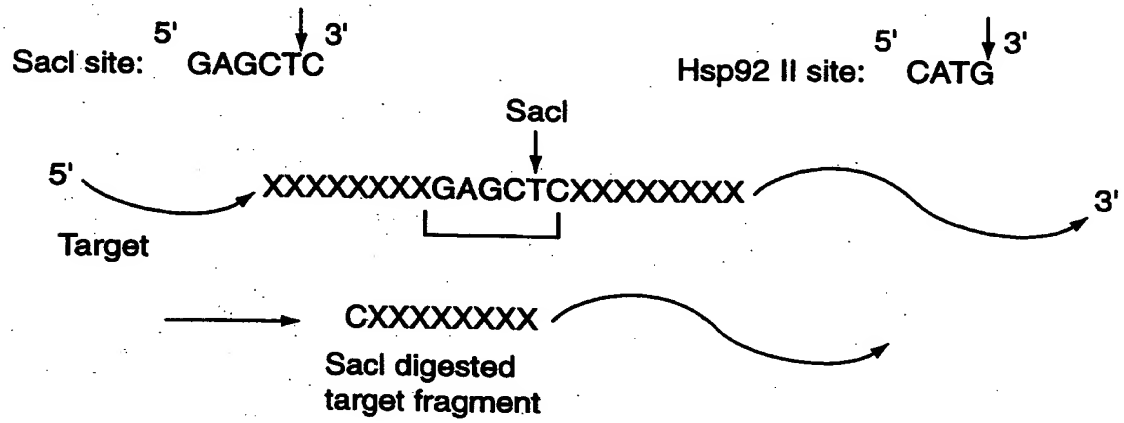


Figure 14b

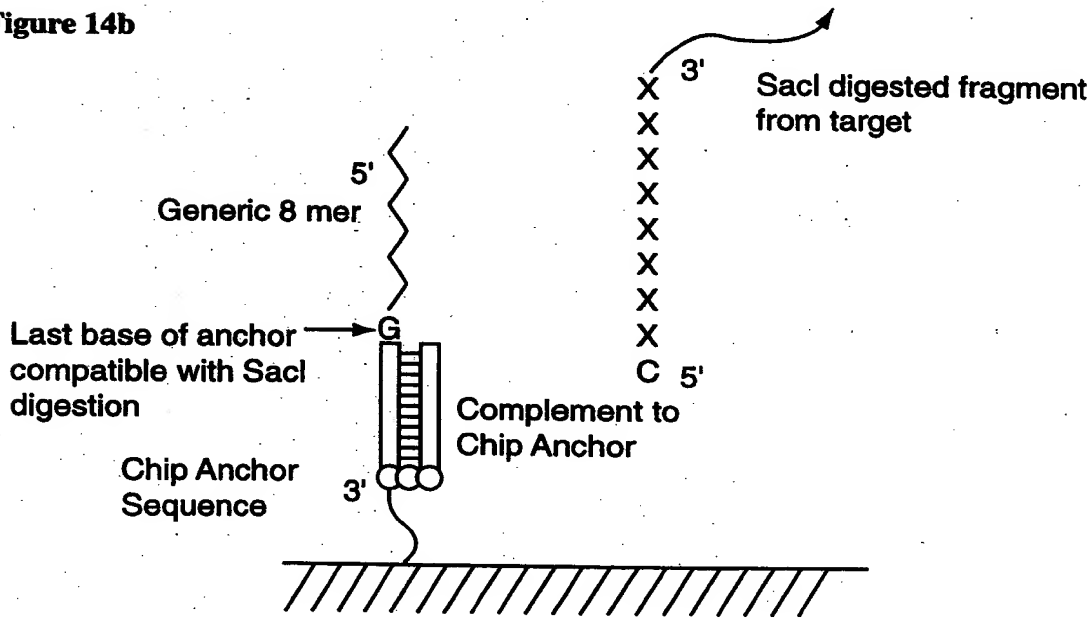
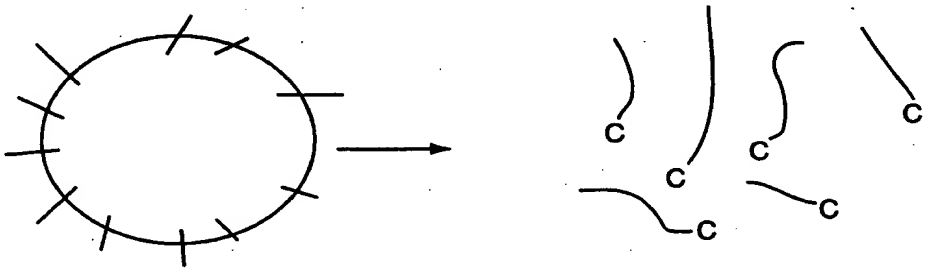


Figure 14c

Monitoring mRNA expression from organisms with small genomes:



6 Mb genome or cDNA library

~ 1 kb genomic or cDNA fragments with 5' C

Figure 14d

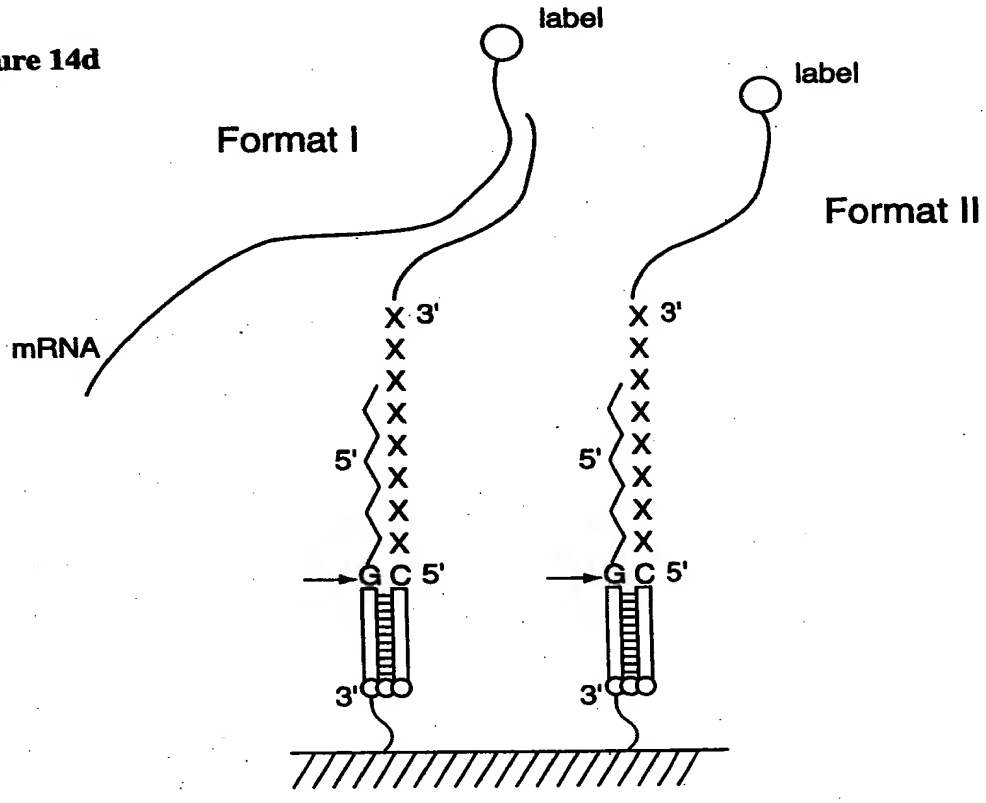


Figure 15a

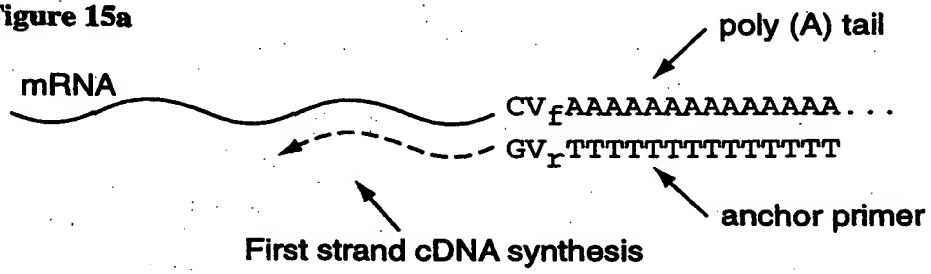


Figure 15b

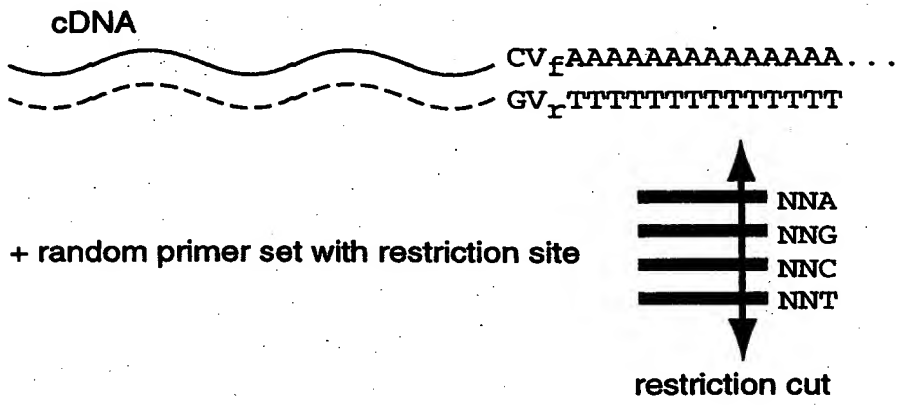


Figure 15c

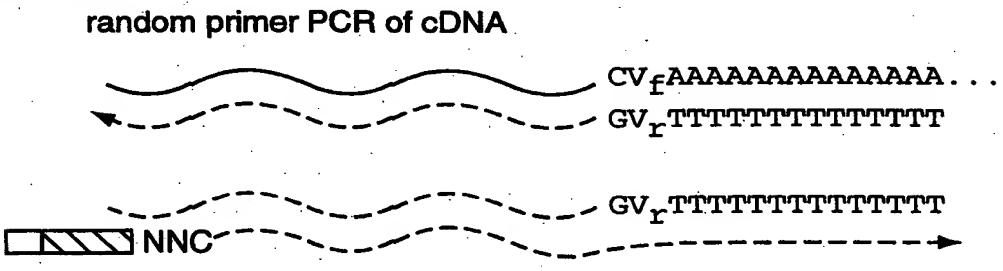


Figure 15d

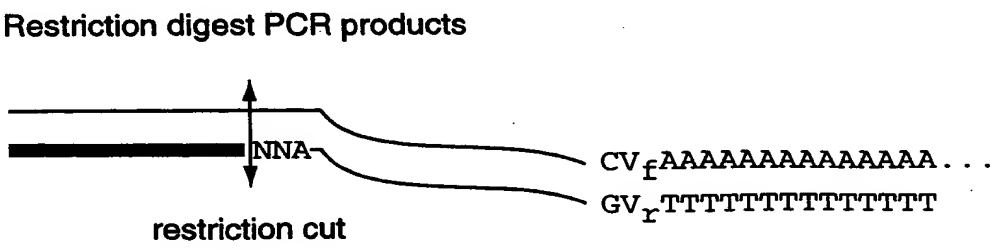
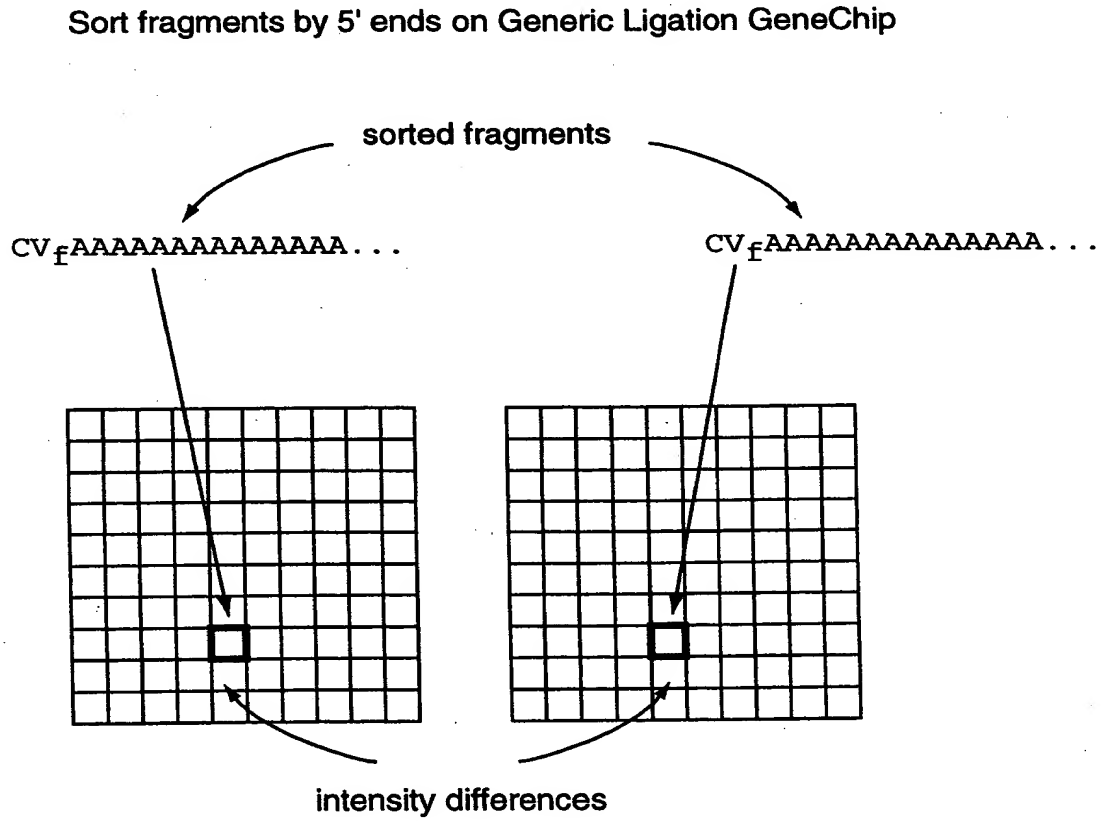
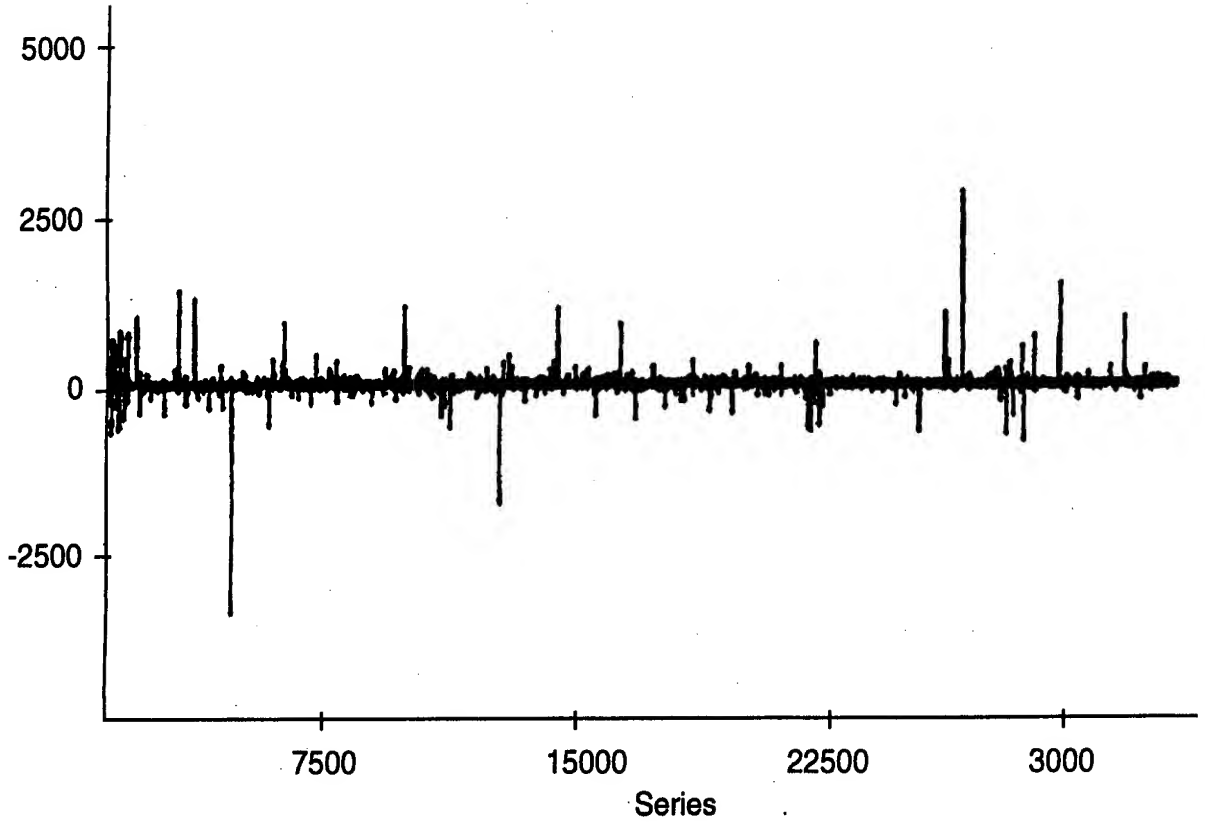


Figure 15e

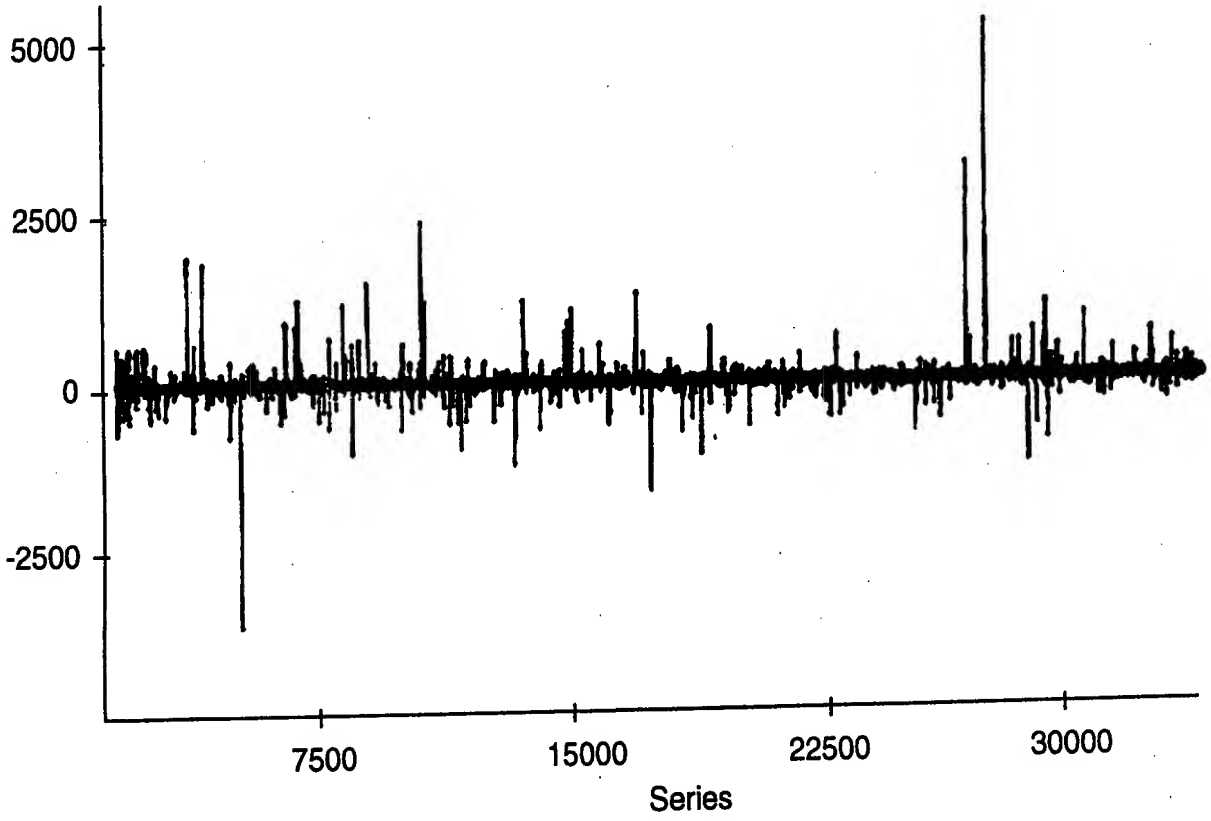


T02T98 22208860



Sample 1 vs. Sample 1 - Absolute Differences
(Replicate 1 vs. Replicate 2)

Figure 16a



Sample 2 vs. Sample 2 - Absolute Differences
(Replicate 1 vs. Replicate 2)

Figure 16b

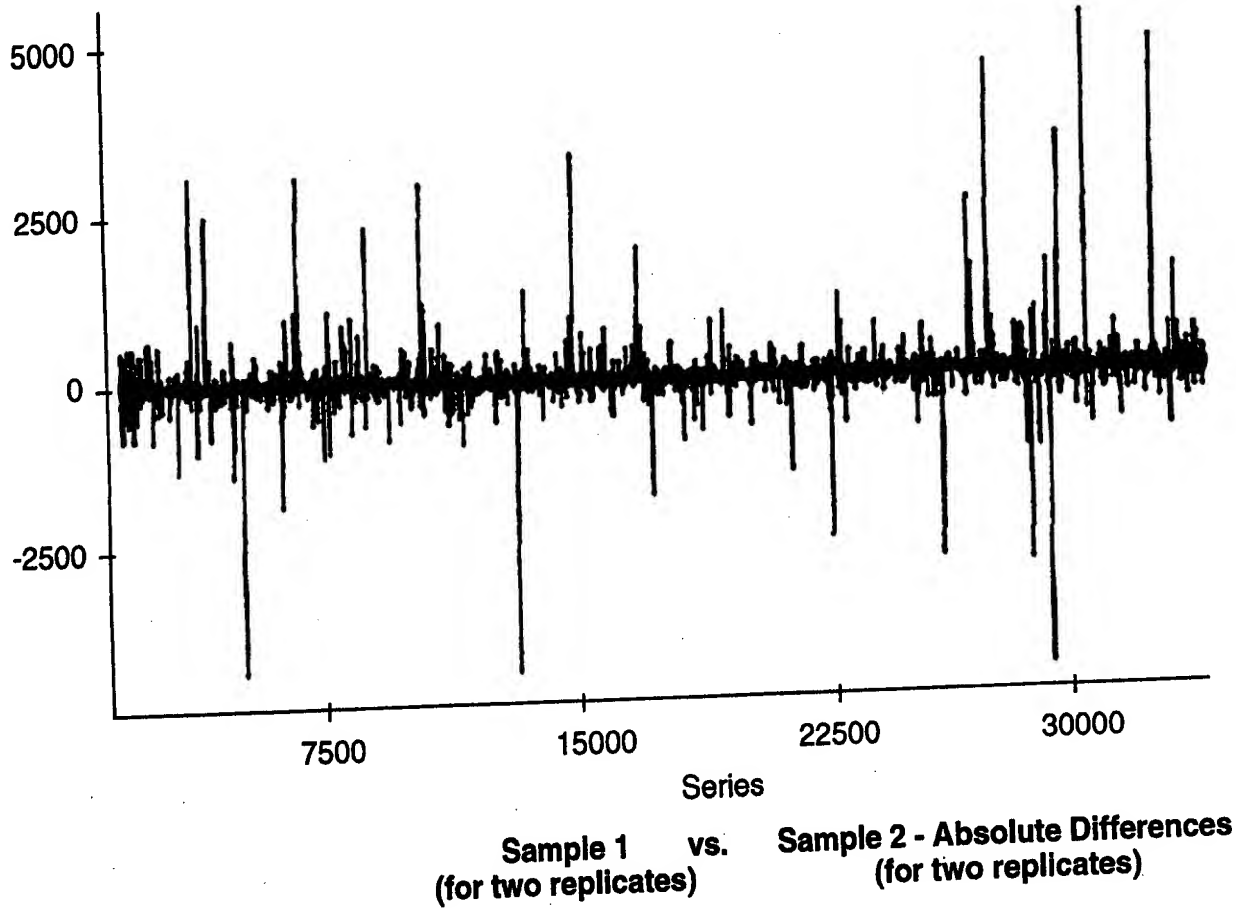

5000
2500
0
-2500
7500
15000
22500
30000
Series
Sample 1 vs. Sample 2 - Absolute Differences
(for two replicates)
(for two replicates)

Figure 16c

T0ET90 22208860

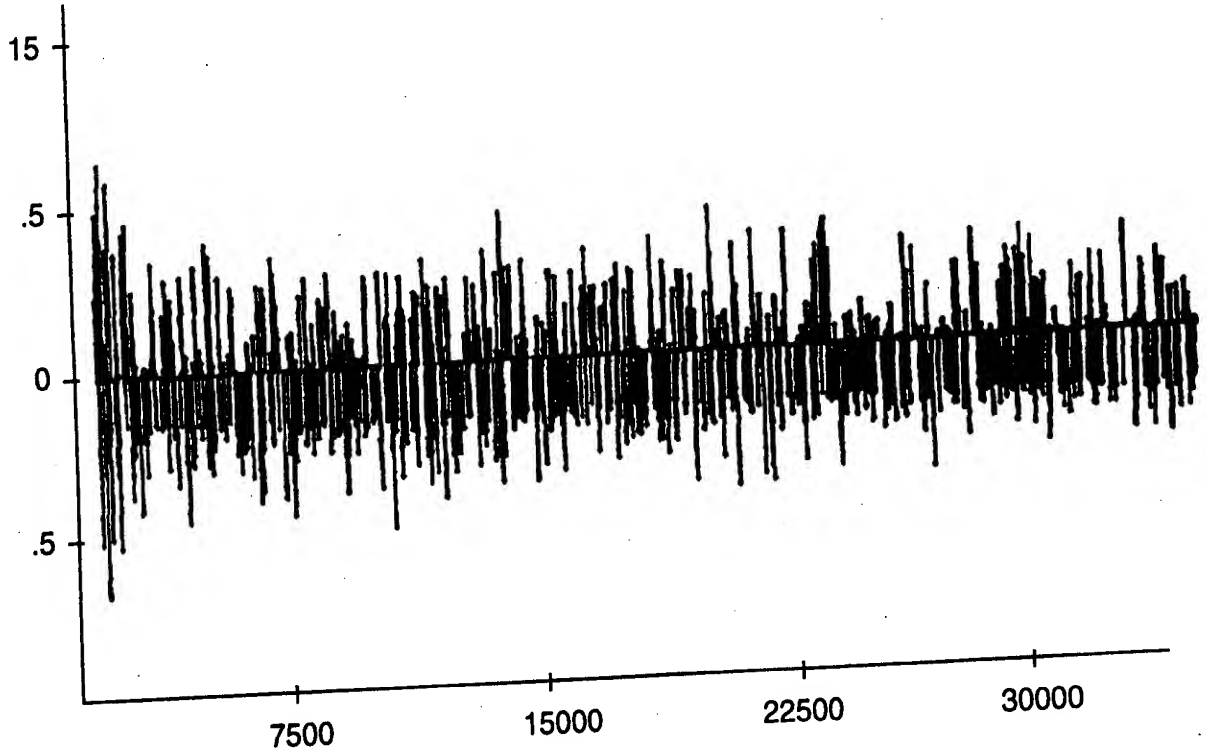
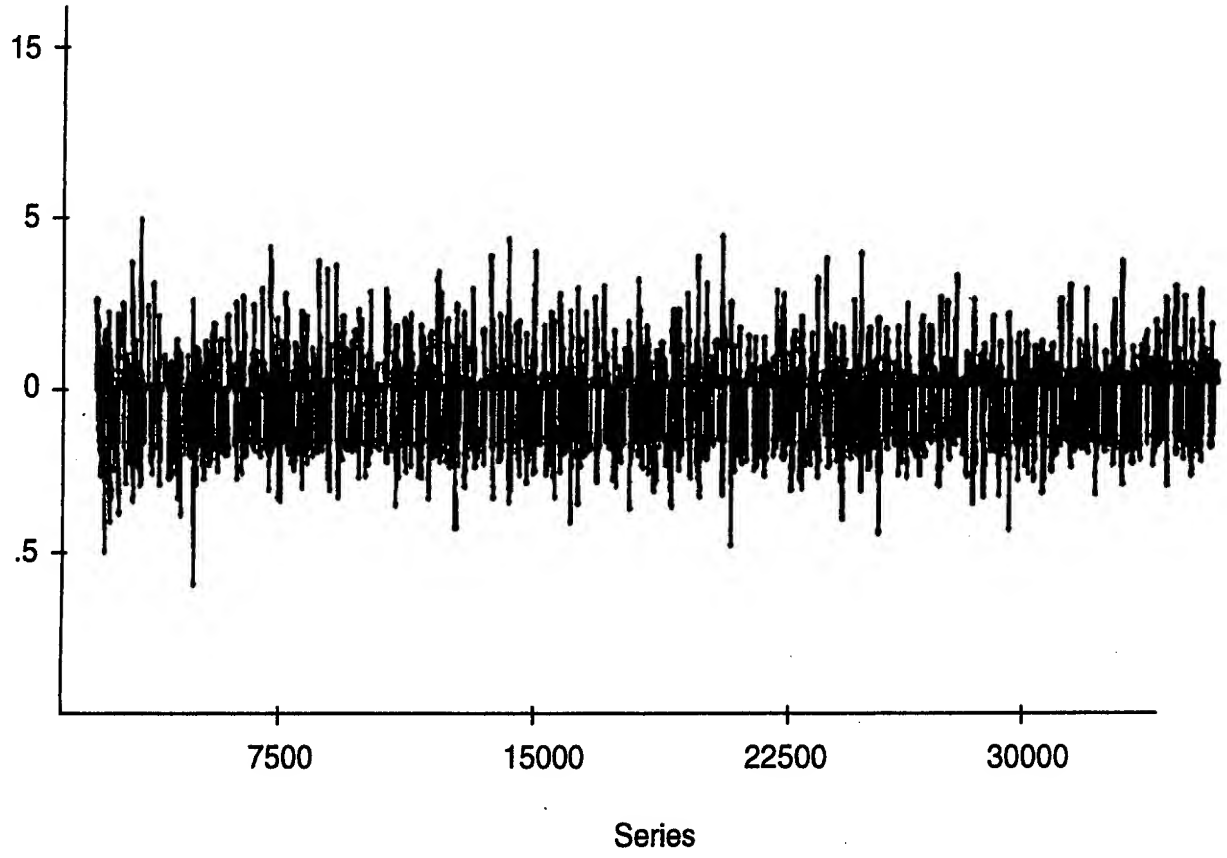


Figure 17a



Sample 2 (Rep 1) vs. Sample 2 - Ratios (Rep 2)

Figure 17b

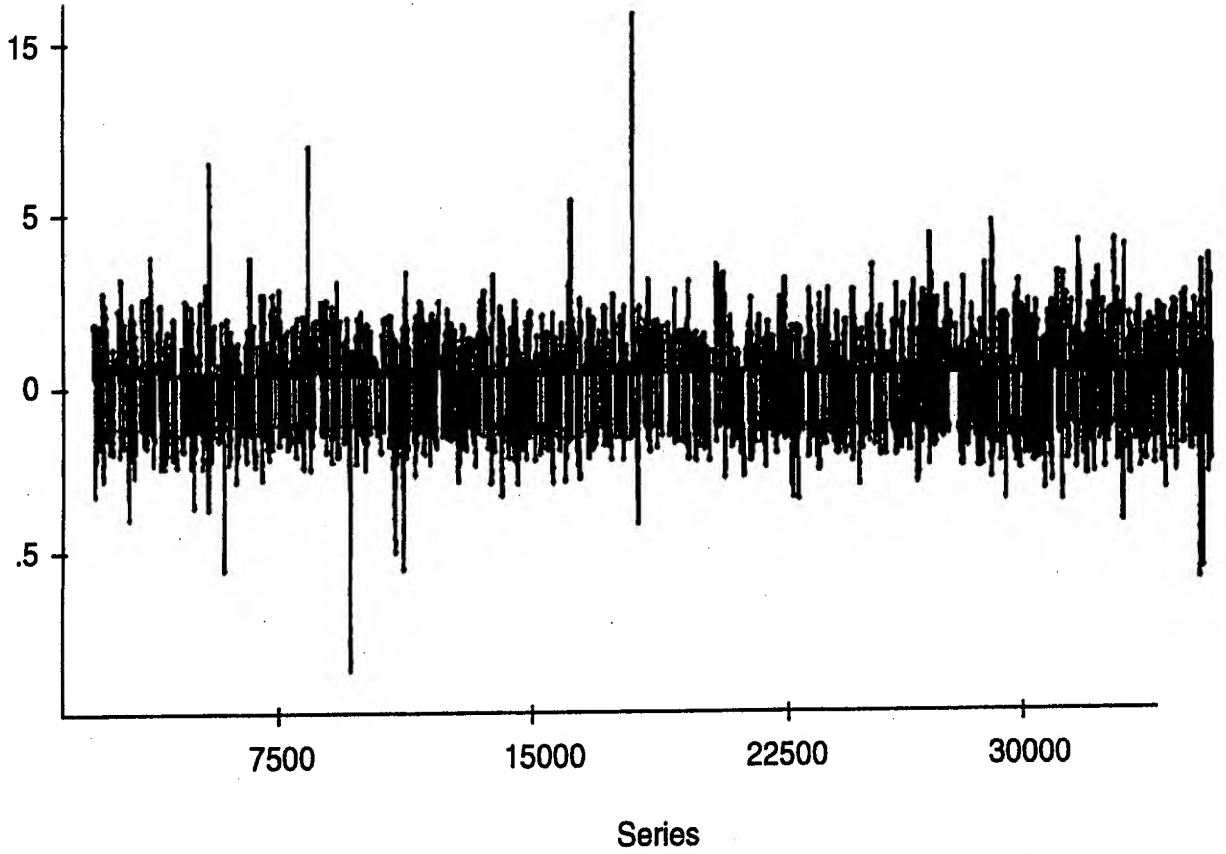
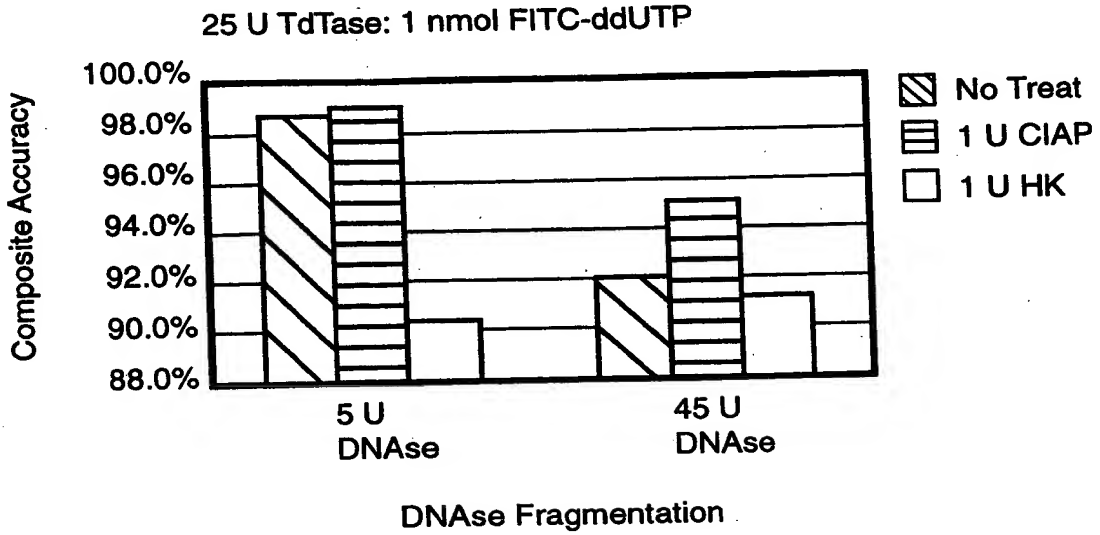

15
5
0
.5
7500
15000
22500
30000
Series
Sample 1 (two replicates) vs. Sample 2 - Ratios (two replicates)

Figure 17c

Post-Fragmentation End Labeling: CIAP Treatment



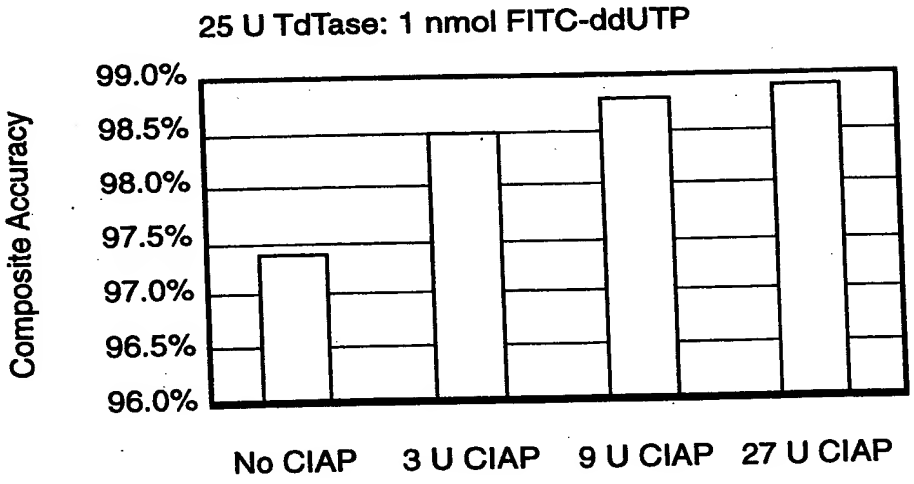


Figure 18

Post-Hybridization End Labeling on the Chip

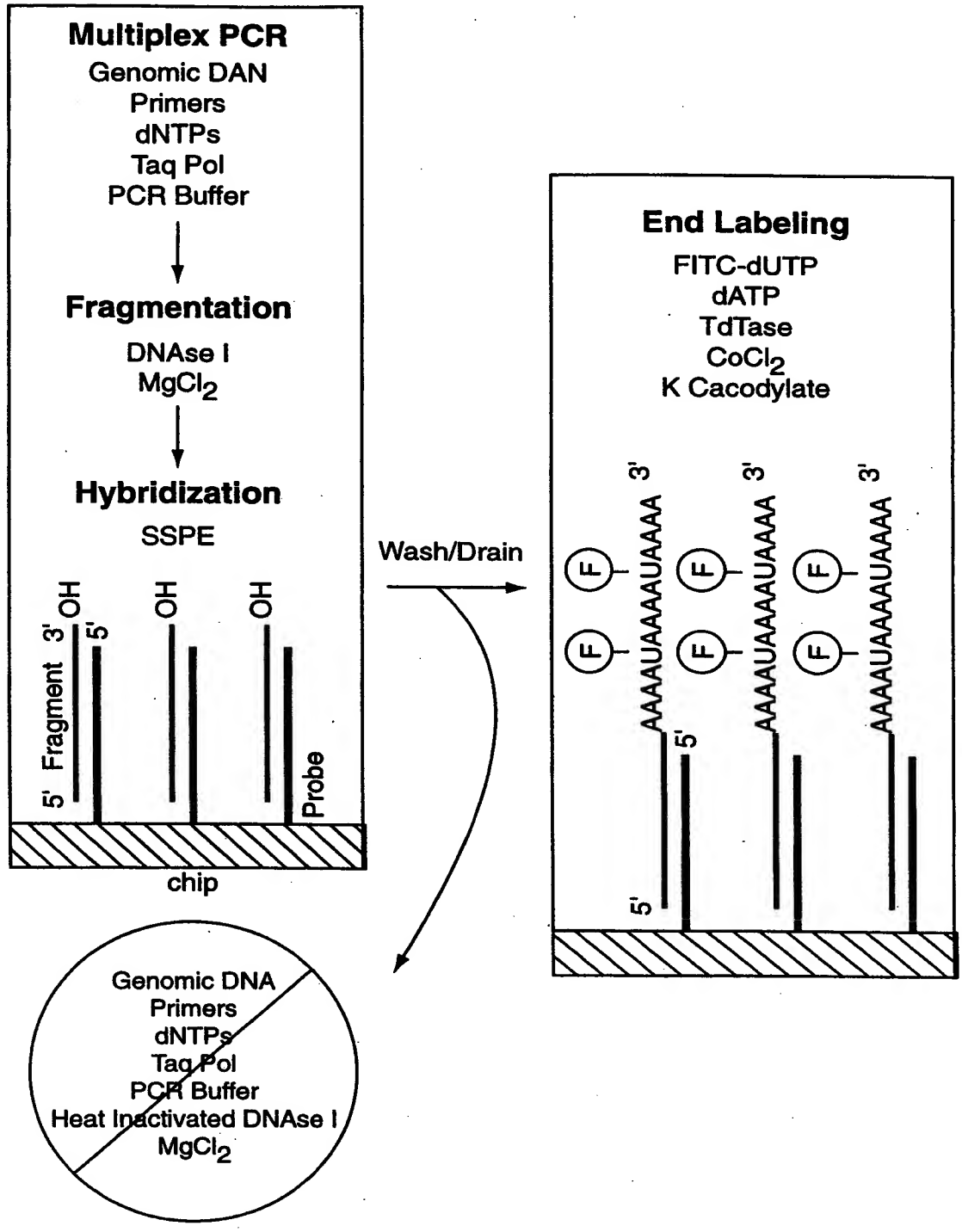


Figure 19

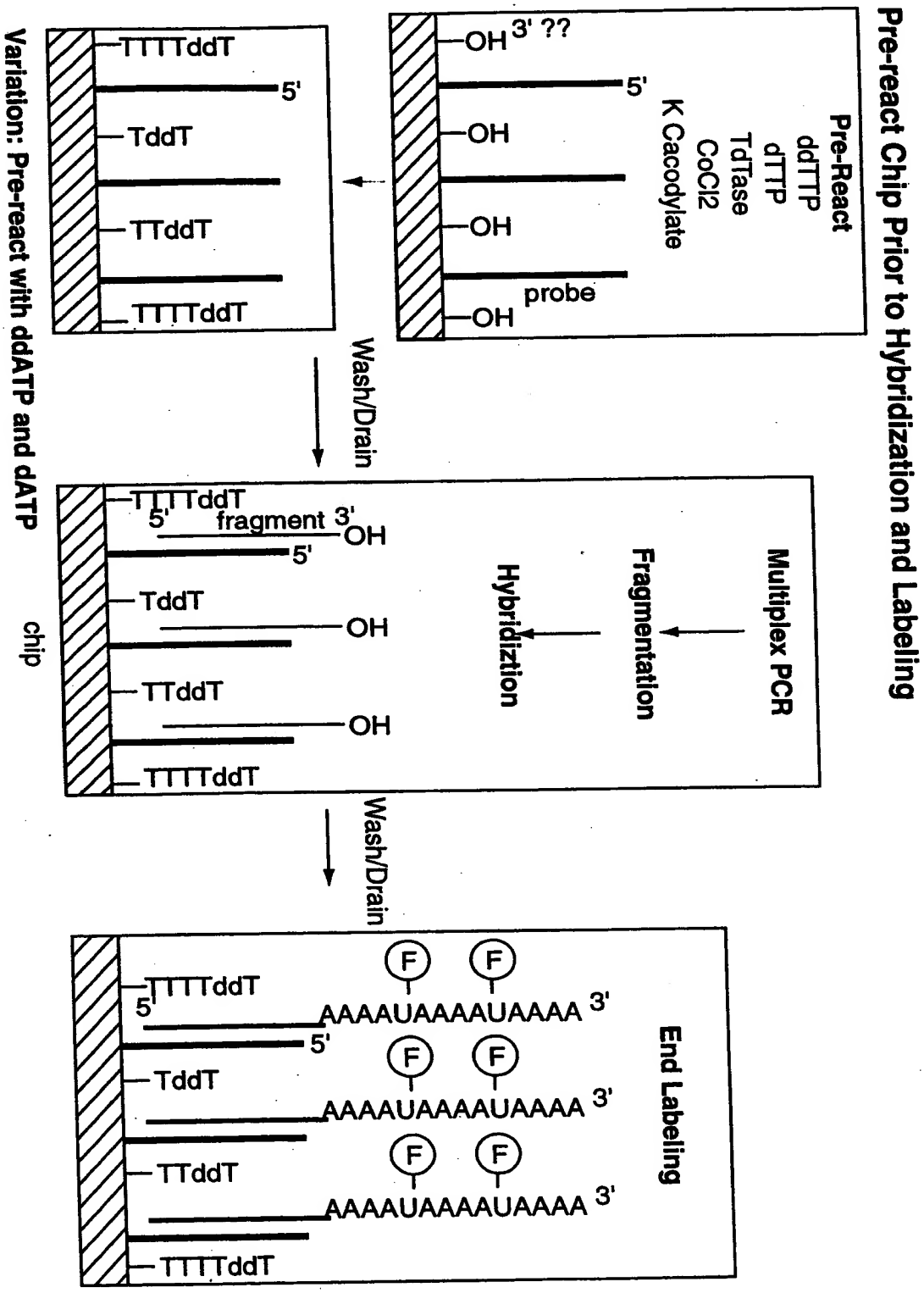

Pre-react Chip Prior to Hybridization and Labeling
Pre-React
ddTTP
dTTP
TdTase
CoCl2
K Cacodylate
OH 3' ??
5'
OH
OH
probe
OH
Wash/Drain
TTTTddT
5'
TddT
TTddT
TTTTddT
Variation: Pre-react with ddATP and dATP
chip
Multiplex PCR
Fragmentation
Hybridiztion
TTTTddT
5' fragment 3'
OH
5'
TddT
OH
TTddT
OH
TTTTddT
Wash/Drain
End Labeling
F
TTTTddT
5'
AAAAUAAAAUAAAA 3'
5'
TddT
AAAAUAAAAUAAAA 3'
TTddT
AAAAUAAAAUAAAA 3'
TTTTddT

Figure 20

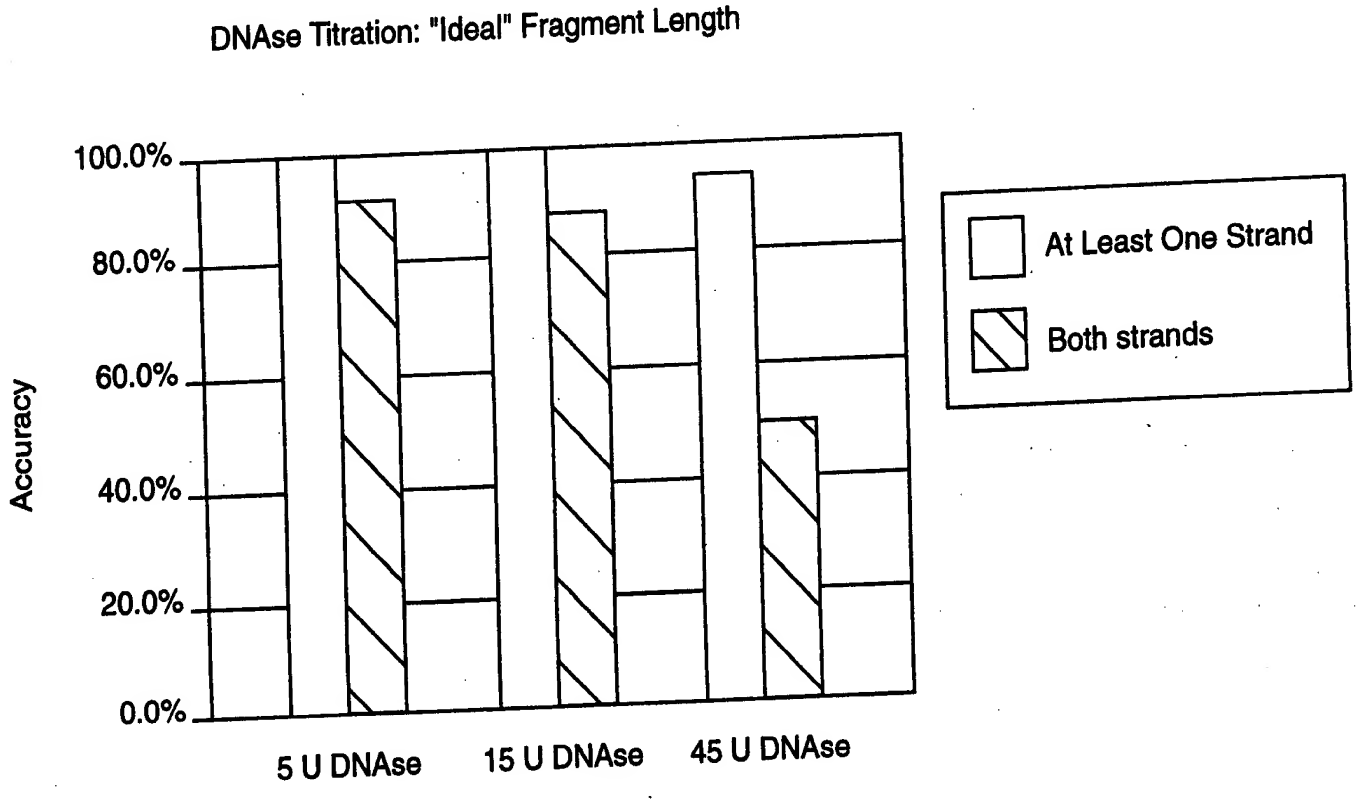


Figure 21

09880727.051301

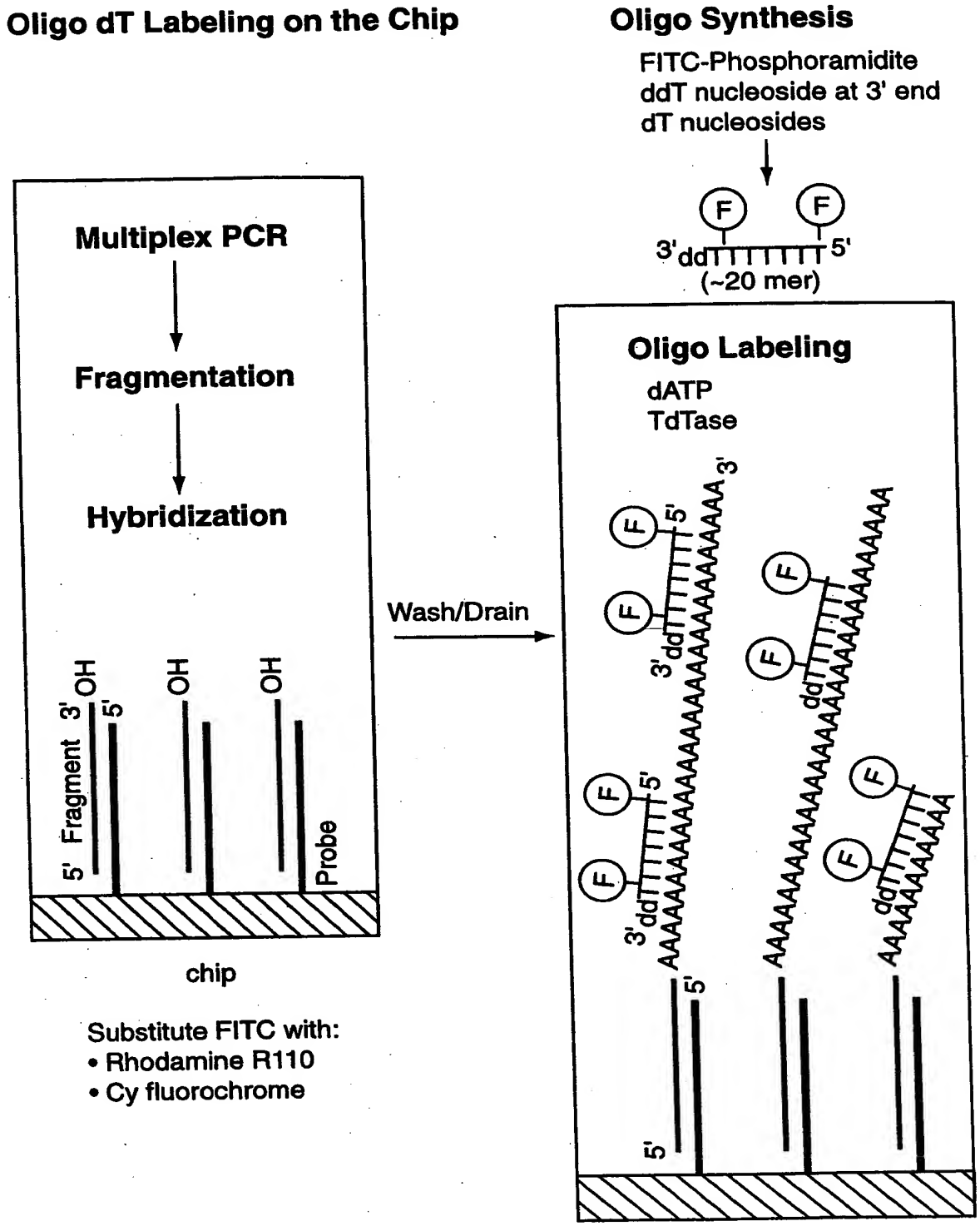

Oligo dT Labeling on the Chip
Oligo Synthesis
FITC-Phosphoramidite
ddT nucleoside at 3' end
dT nucleosides
F
F
3'ddTTTTTTTT 5'
(~20 mer)
Multiplex PCR
Fragmentation
Hybridization
Oligo Labeling
dATP
TdTase
Wash/Drain
5' Fragment 3'OH
5'
OH
OH
Probe
chip
Substitute FITC with:
• Rhodamine R110
• Cy fluorochrome

Figure 22

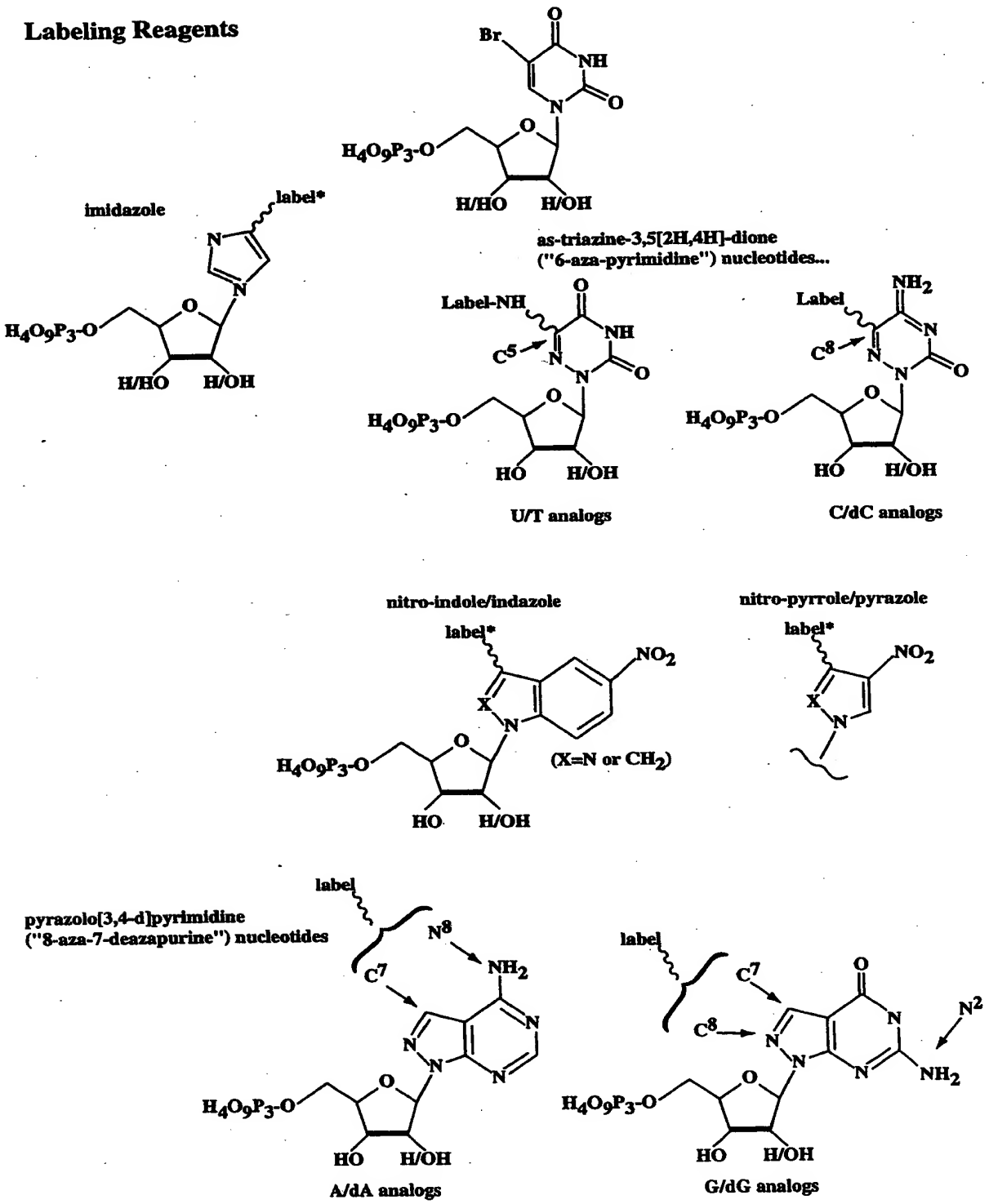

Labeling Reagents
imidazole
label*
as-triazine-3,5[2H,4H]-dione
("6-aza-pyrimidine") nucleotides...
Label-NH
Label
U/T analogs
C/dC analogs
nitro-indole/indazole
nitro-pyrrole/pyrazole
(X=N or CH2)
pyrazolo[3,4-d]pyrimidine
("8-aza-7-deazapurine") nucleotides
A/dA analogs
G/dG analogs

Figure 23a

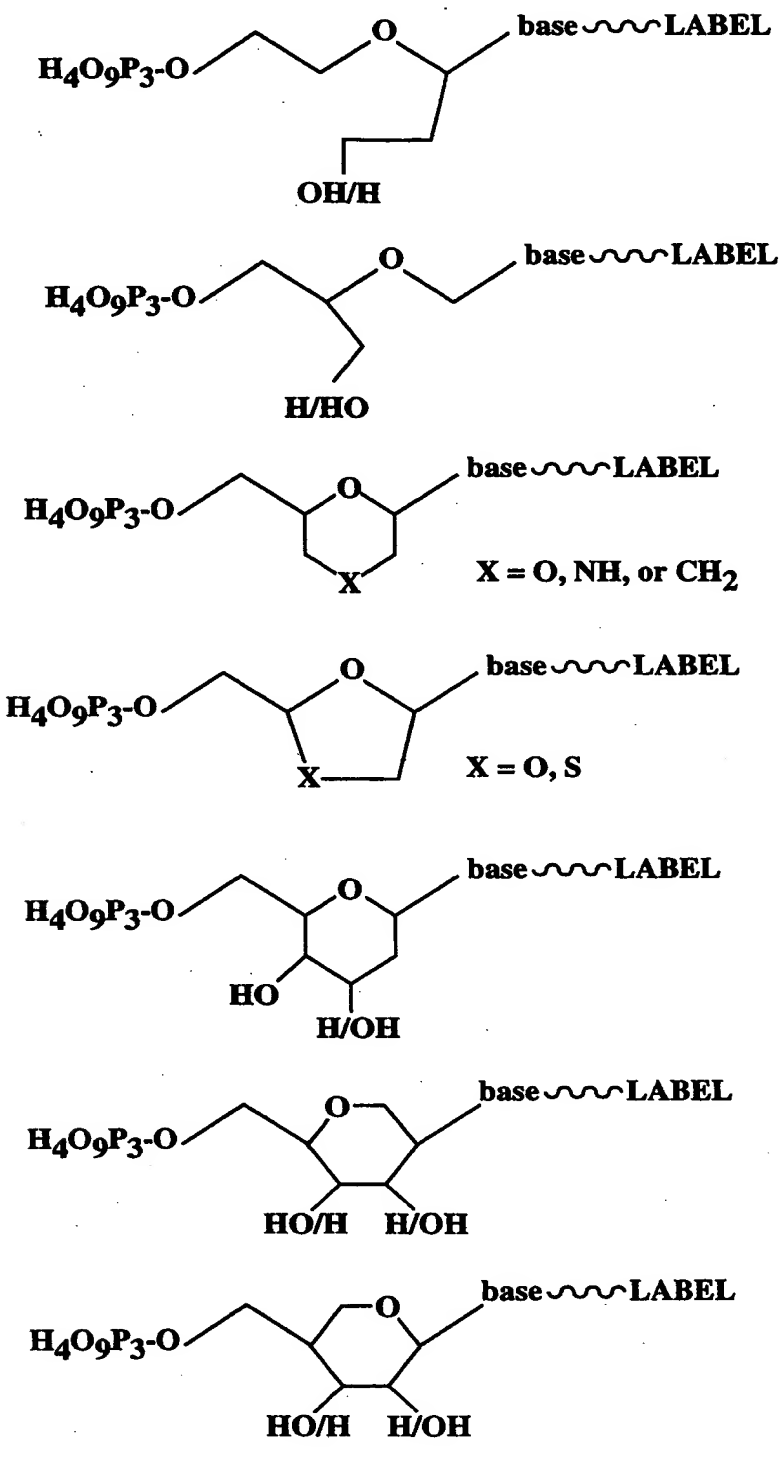
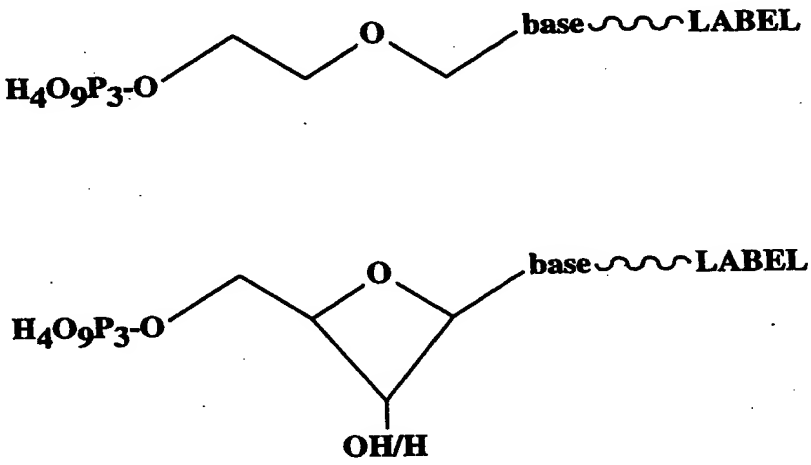


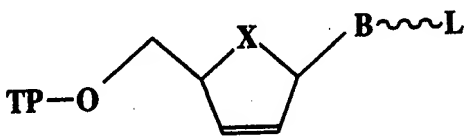
Figure 23b



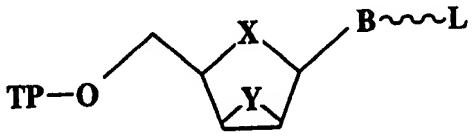
base = heterocycloc moiety (eg. analogs thereof)
∿∿∿ = linker;
LABEL = detectable signal-gene

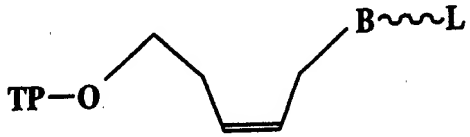
Figure 23c

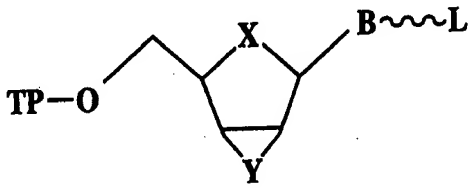
TOCT90"ZZZ08860



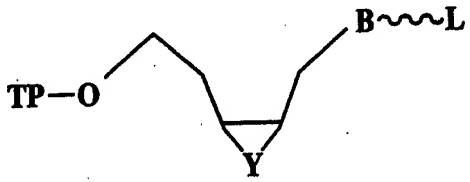
(X = O, S, CH_2)







(X = O, S, CH_2)
(Y = O, S)



(Y = O, S)

L = label
TP = triphosphate

Figure 23d

Resequencing a target DNA molecule with a set of generic n-mer tiling probes

ie. 4-mer probes:

```
                5'                    3'
Target:  TGACATAGGACAGCGAAGGGA...

Probe 1: ACTG 5'
Probe 2:  CTGT
Probe 3:   TGTA
            GTAT
Probe 5:    TATC
             ATCC
              TCCT
               CCTG
Probe 9:        CTGT...etc.
```

Figure 24

Four electronic tiling arrays are present on a 4-mer generic array:

(4 x 3 = 12 "nearest neighbors" for each probe)

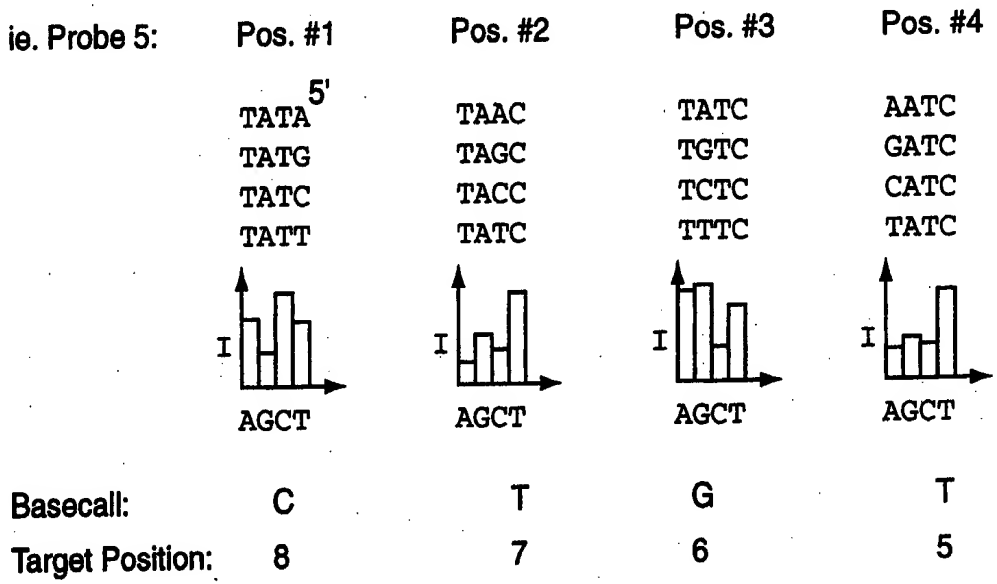


Figure 25

Base Calling at the 8th position in the target

	8th	
Target:	TGACATAGGACAGCGAAGGGA . . .	
	3' 5'	Base-Call
Probe 5, Pos. 1	TATC	T
Probe 6, Pos. 2	ATCC	G
Probe 7, Pos. 3	TCCT	C
Probe 8, Pos. 4	CCTG	C
		C is the winner

Figure 26

Base Vote Table

Base Position	Base Identity	Base Vote	Correctness Score Total		Correctness Score #1	Correctness Score #2	Correctness Score #3	Correctness Score #4
5	T	T	1		1	0	1	0
6	A	A	1		1	1	1	1
7	T	T	1		0	1	0	1
9	C	G	0		0	0	0	0
10	T	T	1		1	0	1	0
11	G	G	1		0	1	1	1
12	T	T	1		0	1	1	1
13	C	C	1		1	0	0	1
14	G	G	1		0	1	1	1
(10) TOTALS								

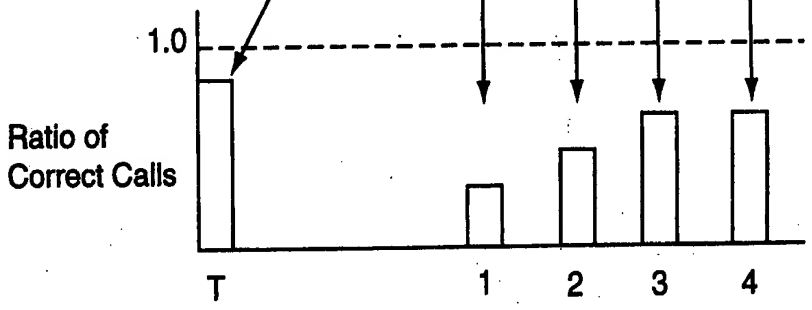


Figure 27

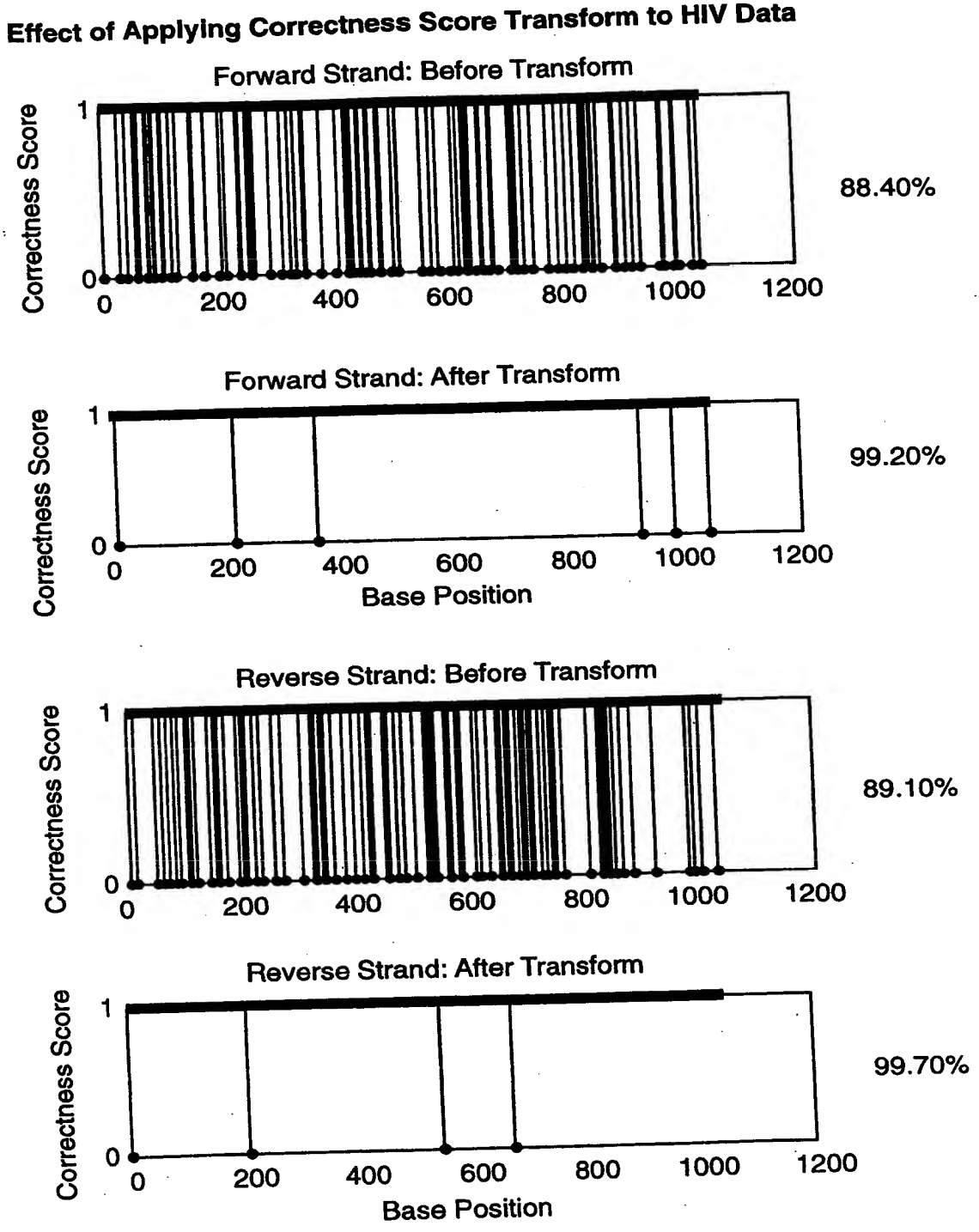
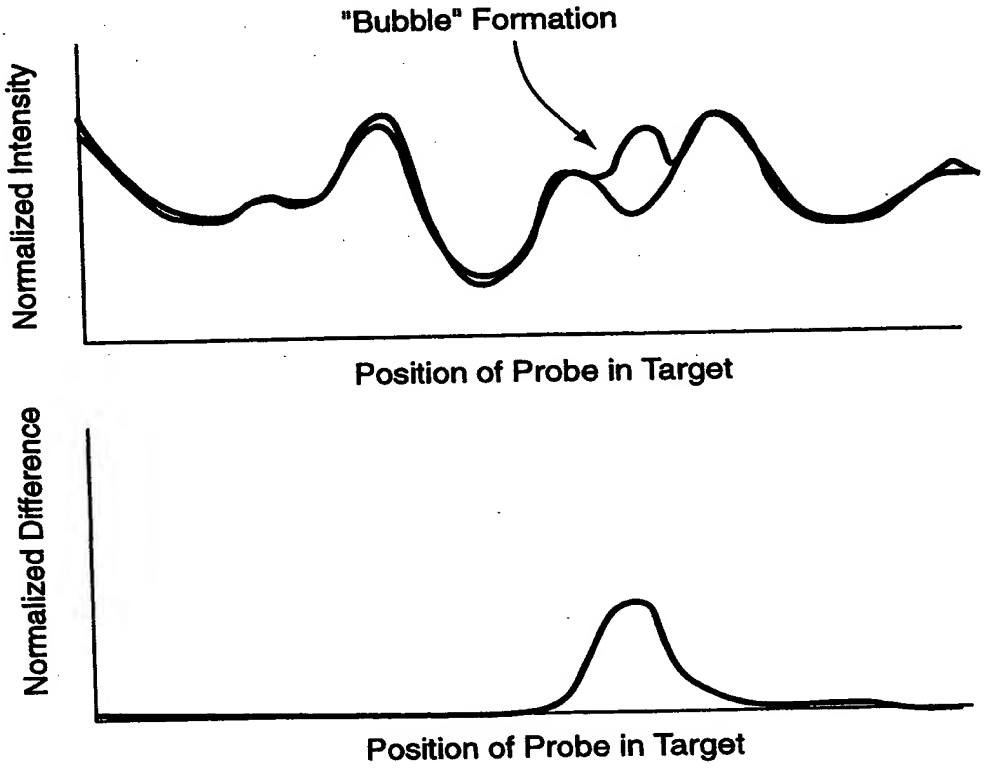

Effect of Applying Correctness Score Transform to HIV Data
Forward Strand: Before Transform
Correctness Score
1
0
0
200
400
600
800
1000
1200
88.40%
Forward Strand: After Transform
Correctness Score
1
0
0
200
400
600
800
1000
1200
Base Position
99.20%
Reverse Strand: Before Transform
Correctness Score
1
0
0
200
400
600
800
1000
1200
89.10%
Reverse Strand: After Transform
Correctness Score
1
0
0
200
400
600
800
1000
1200
Base Position
99.70%

Figure 28

Mutation Detection by Intensity Comparisons



Algorithms:

$$I_{normalized} = I_{probe}/(\Sigma I_{NN})$$

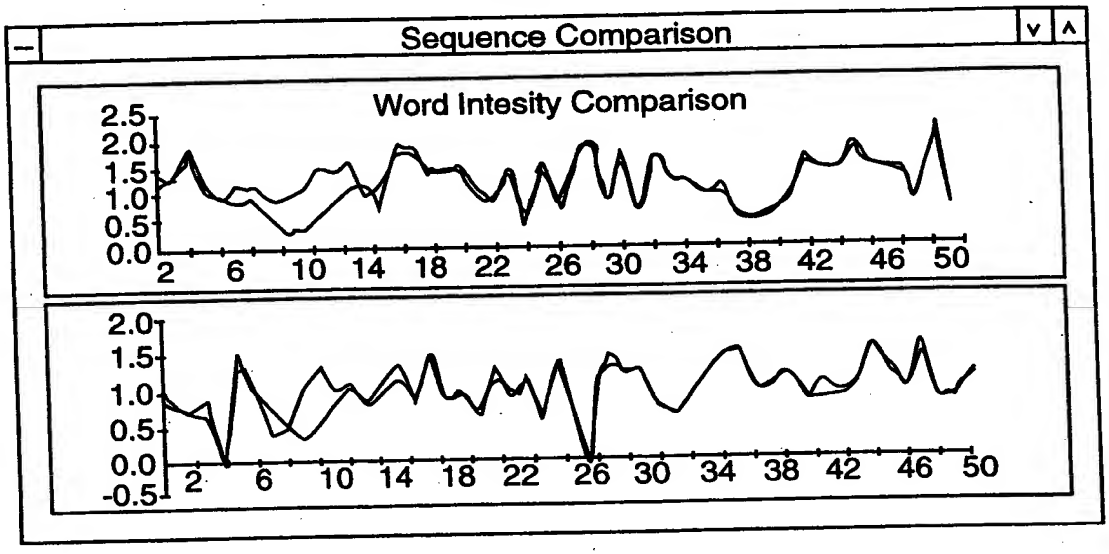
$$I_{difference} = \frac{(I_{normalized,\ variant} - I_{normalized,\ control})}{(I_{normalized,\ variant} + I_{normalized,\ control})}$$

- Locally normalized intensities track well
- Local normalization is sensitive to mutations

Figure 29

Bubble Formation Detection of Mutation in HIV Genome

Normalized Intensity Comparison:



Normalized Difference:

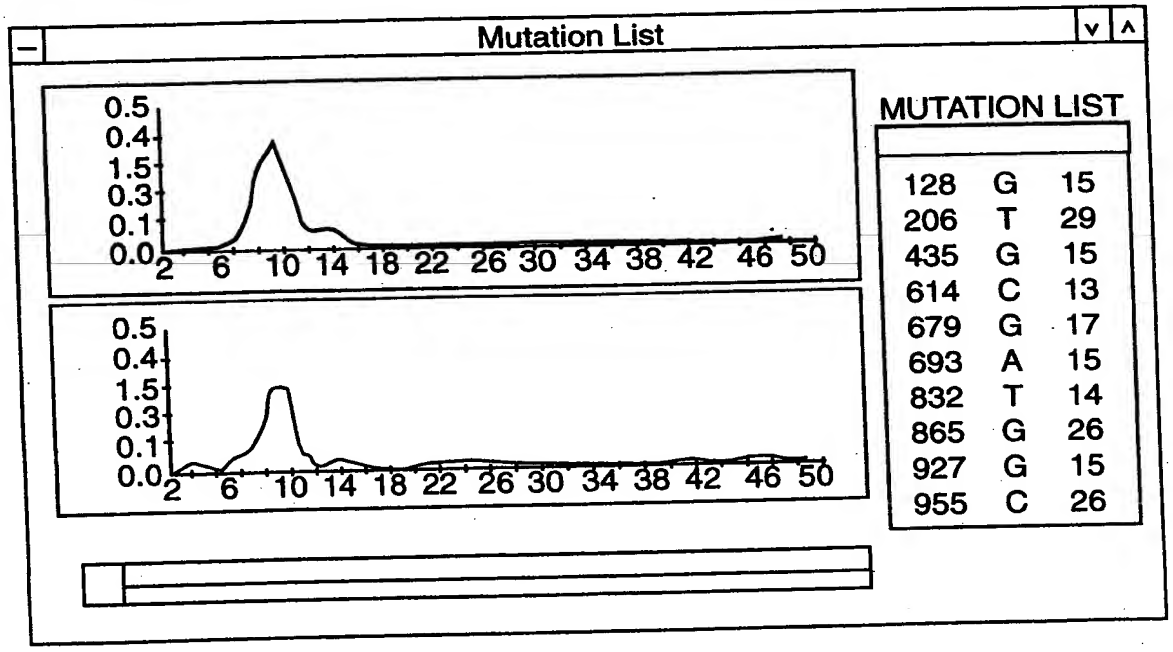
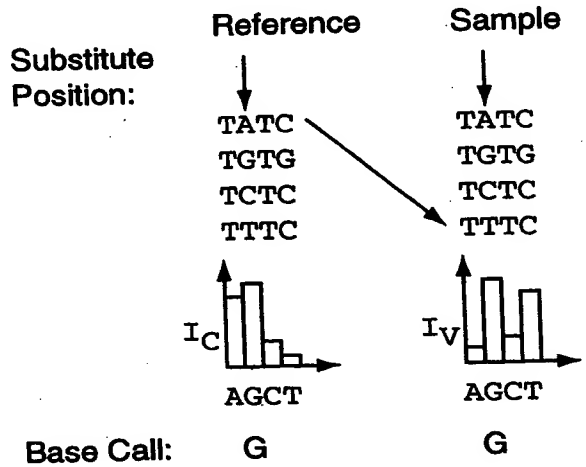


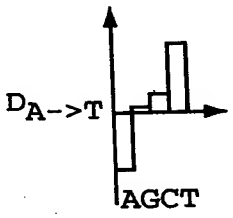
Figure 30

Induced Difference Nearest Neighbor Probe Scoring:



Induced Difference: $D_A = (I_{V,A} - I_{C,A}) / I_{C,A}$

- Average induced differences over all tilings and over both forward and reverse strands.



- Probe with A - "down-regulated"
- Probe with T - "up-regulated"
- A ---> T mutation

- Total Induced Difference > + Threshold:Mutation Exists
  Total Induced Difference < - Threshold:Mutation Exists
- Two criteria for mutations: Induced Difference Scores; Bubble Formation

Figure 31

Mutations found in an HIV PCR target (B) using a generic ligation GeneChip and induced difference analysis

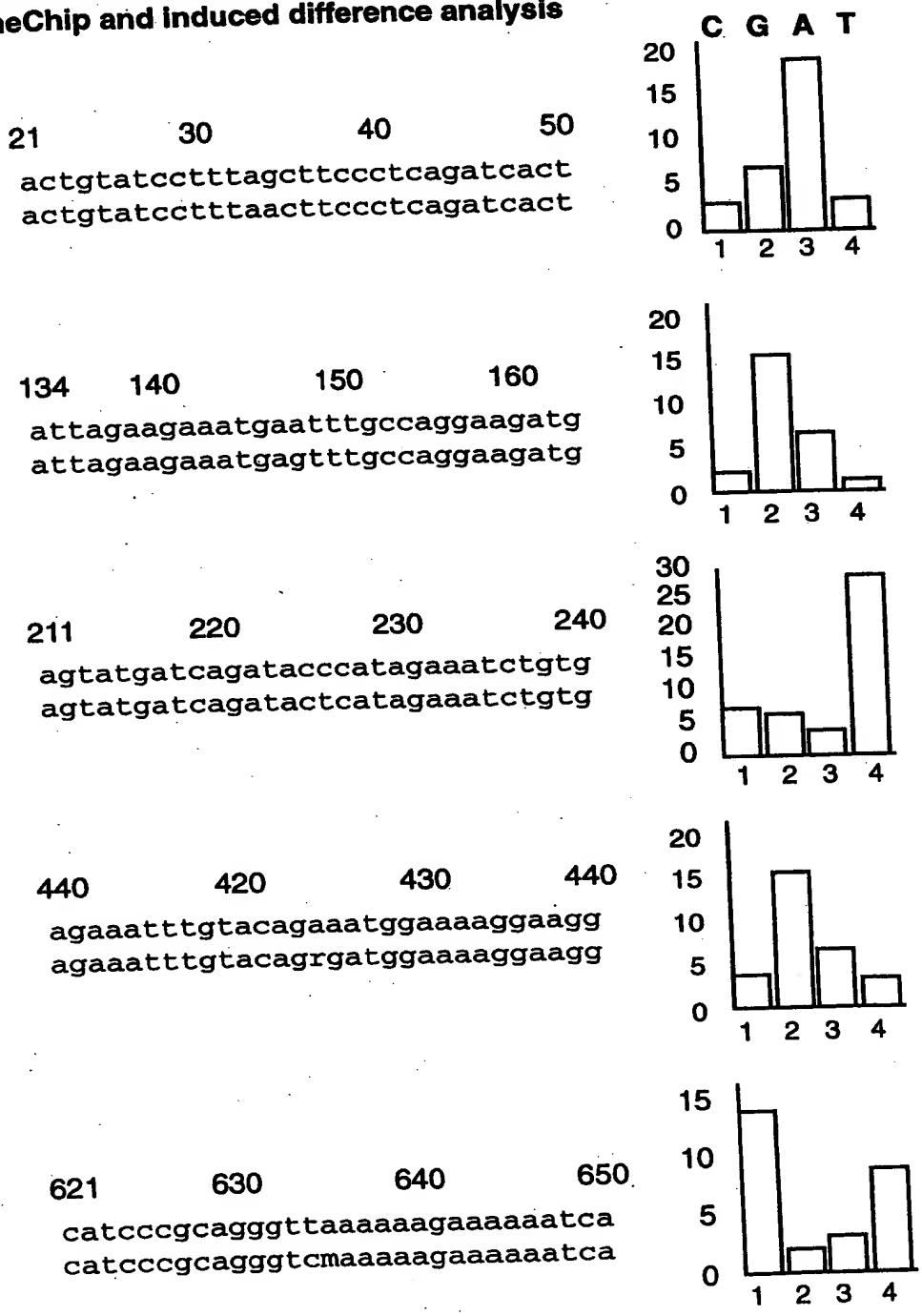


Figure 32a

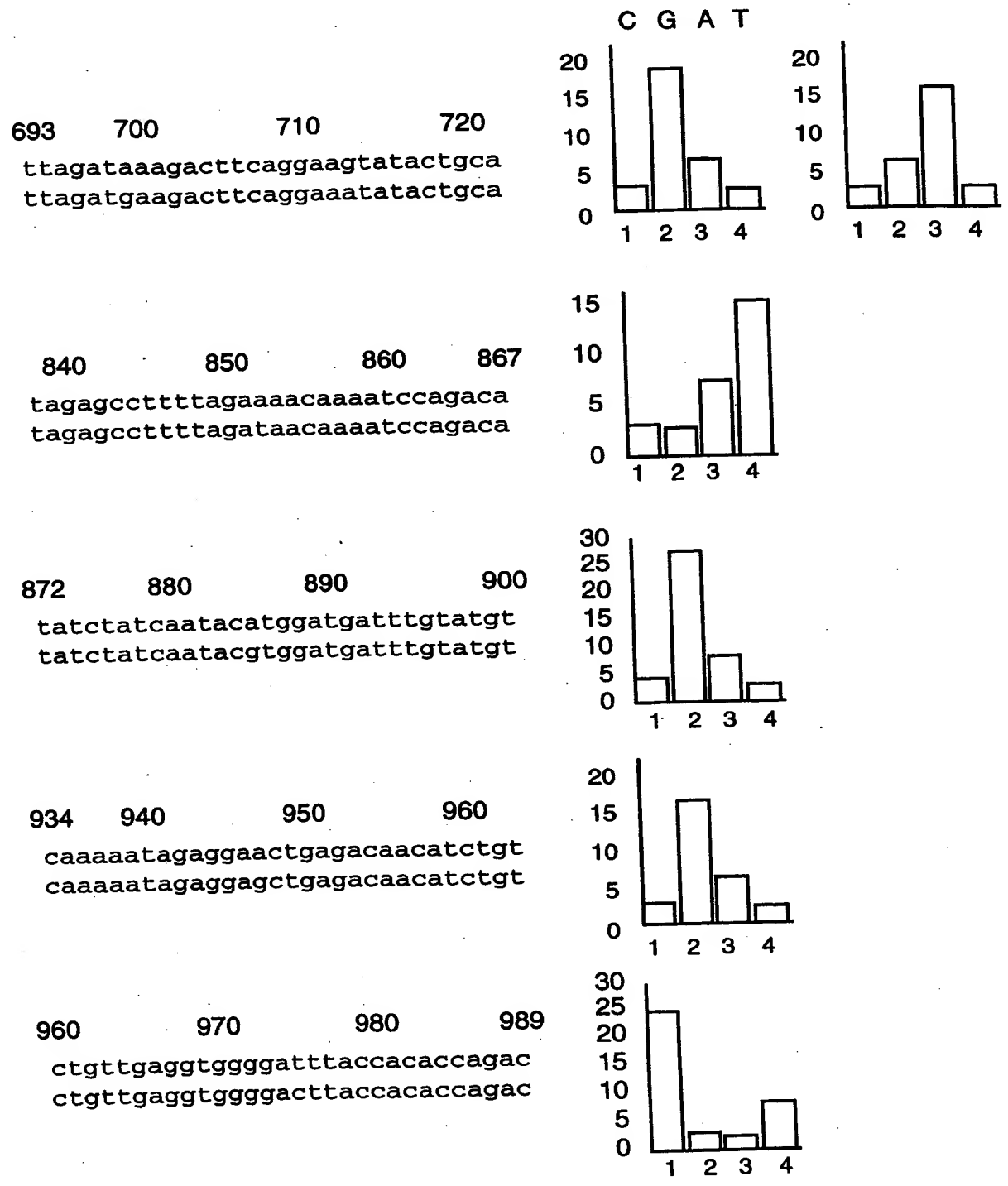
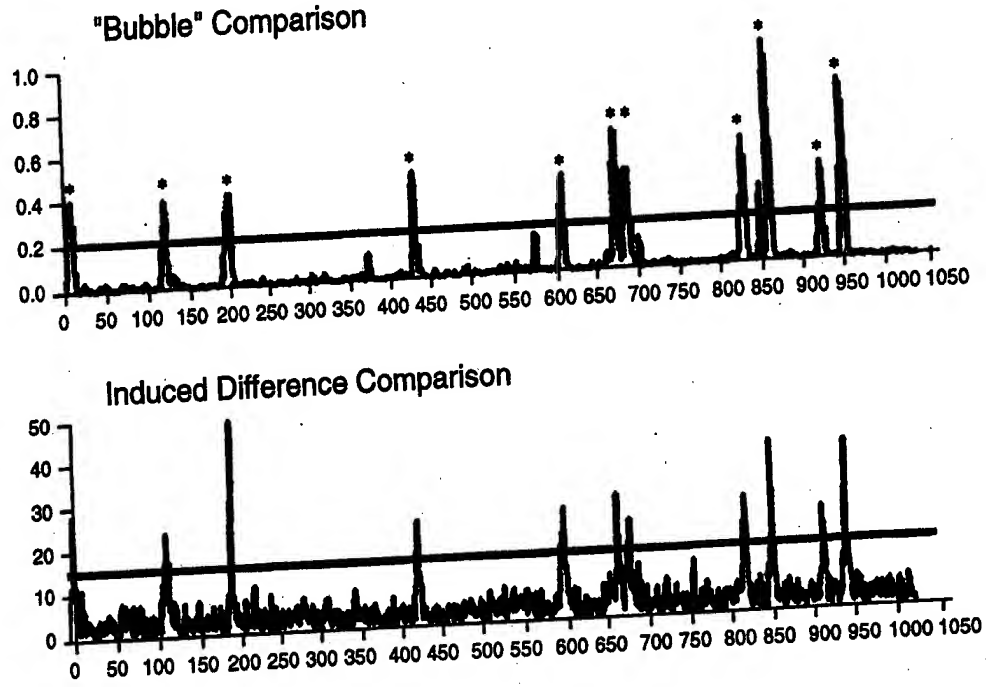

C G A T
693 700 710 720
ttagataaagacttcaggaagtatactgca
ttagatgaagacttcaggaaatatactgca
840 850 860 867
tagagcctttagaaaacaaaatccagaca
tagagcctttagataacaaaatccagaca
872 880 890 900
tatctatcaatacatggatgatttgtatgt
tatctatcaatacgtggatgatttgtatgt
934 940 950 960
caaaaatagaggaactgagacaacatctgt
caaaaatagaggagctgagacaacatctgt
960 970 980 989
ctgttgaggtggggatttaccacaccagac
ctgttgaggtggggacttaccacaccagac

Figure 32b

Mutation Detection Using Comparisons Between a Reference Target and a Sample Target



Results: No false positives, all 11 mutations (indicated by *) are detected in this 1041 bp HIV DNA fragment.

Figure 33